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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>A61L 27/00</b>		A1	(11) International Publication Number: <b>WO 97/42987</b> (43) International Publication Date: 20 November 1997 (20.11.97)
(21) International Application Number: <b>PCT/IL97/00154</b> (22) International Filing Date: 8 May 1997 (08.05.97)		(81) Designated States: AI, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(30) Priority Data: 118235 13 May 1996 (13.05.96) IL		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
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(54) Title: COMPOSITION AND METHOD FOR FORMING BIODEGRADABLE IMPLANTS <i>IN SITU</i> AND USES OF THESE IMPLANTS			
(57) Abstract			
<p>The invention discloses a composition comprising a mixture of a pharmaceutically, medically or veterinarianily acceptable polymer, preferably a poly(lactic-co-glycolic acid) copolymer (PLGA) containing between about 10 and 100 wt.% lactic acid (LA) units, preferably between about 50 and 90 wt.% LA units, and <math>\alpha</math>-(tetrahydrofuranyl)-<math>\omega</math>-hydroxypoly(oxy-1,2-ethanediyl) (glycofurof). Methods of forming solid implants <i>in situ</i> in an animal body, said implants optionally comprising a drug or other biologically active agent, as well as the use of the compositions of the invention in the treatment of animal bodies, are also disclosed.</p>			

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**COMPOSITION AND METHOD FOR FORMING**  
**BIODEGRADABLE IMPLANTS IN SITU AND USES OF THESE**  
**IMPLANTS**

5    **Field of the Invention**

This invention relates to a composition useful in the *in situ* formation of biodegradable polymeric implants of polymers and copolymers of lactic and glycolic acid, to a method of forming such implants *in situ* using the composition of the invention, to implants formed thereby, and to uses of 10 such composition, method or implants as a space-filler, for soft or hard tissue regeneration, and for the controlled release of drugs.

**Background of the Invention**

The use of biodegradable polymers in medical applications, such as 15 sutures, staples, surgical clips, implant and drug delivery systems, is well known. A particular use is the *in situ* formation of biodegradable implants or inserts; in this application, the term "biodegradable implant" and "biodegradable insert" will be used interchangeably. The *in situ* formation of biodegradable implants is described, for example, in US Patent No. 20 4,938,763; Duysen et al., Pharmaceutical Research, 1994, Amer. Assoc. of Pharm. Scientists, Presentation #7575; Frank et al., Pharmaceutical Research, 1994, Amer. Assoc. of Pharm. Scientists, Presentation #2070;

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Dunn et al., Proc. Int. Symp. Control. Rel. Bioact. Mater., 22 (1995); Dunn et al., Portland Bone Symposium, August 2-5, 1995, Portland, Oregon; Andreopoulos, Clinical Materials 15 (1994) 89-92; Lambert & Peck, J. Controlled Release 33 (1995) 189-195; Shah et al., J. Controlled Release 5 27 (1993) 139-147; Shively et al., J. Controlled Release 33 (1995) 237-243; Lowe et al., 19th Ann. Mtg. Soc. Biomaterials.

Such implants serve two main purposes: as space-filling material, e.g. where tissue has been removed or where bone regeneration is required; 10 and as a mechanism for controlled release of drugs. The advantages of forming such inserts *in situ* as opposed to outside the body are described in the foregoing references, and include the ability to insert the implant without resorting to surgery, as well as the capability of the implant to be formed exactly to the dimensions of the cavity being filled when space- 15 filling is the goal of the implant.

As described in US 4,938,763, one of the methods (the "thermoplastic system") which may be employed for the *in situ* formation of implants is the injection of a solution containing a water-immiscible biodegradable 20 polymer and a water-miscible biologically compatible (non-toxic) solvent into an animal. The solvent is quickly carried away from the injection site, and the polymer left behind in the aqueous environment of the body

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quickly coagulates or solidifies into a solid matrix structure. If the implant is meant to serve as a drug-delivery system, then the drug is incorporated into the solution prior to injection, and is trapped in the solid matrix formed upon coagulation of the polymer. As will be appreciated by persons skilled in the art, different degrees of coagulation, and thus different rates of biodegradation and/or, if applicable, drug release, may be achieved, by varying the characteristics of the polymer or copolymer (e.g., degree of hydrophobicity or average molecular weight), the solvent, and the relative amount of each component prior to injection. The relative amount of the drug and the identity of the drug are also important factors when the implant serves as a controlled-release device.

The polymers and copolymers known in the art to be suitable for use in the "thermoplastic method" of *in situ* implant formation disclosed in US 4,938,763 include poly(L-lactic acid), poly(D-lactic acid), poly(DL-lactic acid), poly(L-lactide), poly(D-lactide), poly(DL-lactide), poly(DL-lactide-co-glycolide), poly(lactic-co-glycolic) acid, polyglycolide, and polyglycolic acid. Solvents known in the art include N-methyl pyrrolidone, propylene glycol, triacetin, triethyl citrate, and dimethyl sulfoxide. As stated, it will be appreciated that the degree of coagulation of the polymer, and thus the rate of biodegradation and/or drug release, is dependent in part on the choice of solvent used. On the other hand, the ability of a given

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biocompatible solvent to dissolve a given biodegradable polymer, and to provide a solution that can be used in a method for the *in situ* formation of a biodegradable polymeric implant is, at best, unpredictable. Not every biocompatible solvent can be used with any given biodegradable polymer,  
5 and not every biodegradable polymer can be used with any given solvent to provide the desired solution for use in *in situ* formation of a biodegradable polymeric implant. Furthermore, in view of the fact that, ultimately, the above biocompatible solvent-biodegradable polymer containing solutions are intended for administration in humans, it is  
10 essential that such solutions be acceptable pharmacologically, i.e., that their administration will be essentially harmless to the patient. Likewise, in veterinary medicine, when such implants are to be formed in, for example, domestic animals, it is essential that the solutions administered to the animals are essentially harmless.

15

Accordingly, in view of the above-mentioned medical and veterinary considerations, the number of potential biocompatible solvent-biodegradable polymer combinations useful for administration to humans and/or animals for *in situ* biodegradable implant formation is considerably  
20 restricted. One of the drawbacks of the prior art noted above is the general failure to provide specific, harmless solutions that can be used for the *in situ* formation of biodegradable polymers; often, at least some of the

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polymers to be dissolved therewith are not suitable for human or animal administration.

Another drawback of the above prior art is that often, when a desirable

5 solvent-polymer combination is obtained that is fit for medical and/or veterinary use, the process by which such a combination, i.e., solution, is produced is often tedious, requiring special conditions for production. For example, in the above-noted US 4,938,763, there are described various methods for preparing some solvent-polymer combinations for use as *in*

10 *situ* forming biodegradable implants, many of which methods require the use of catalysts, more than one solvent, high temperatures and other special conditions to provide the solvent-polymer solution to be administered.

15 Poly(lactic-co-glycolic acid) copolymer (PLGA) is used for injection in man and used for parenteral applications. Glycofurool ( $\alpha$ -(tetrahydrofuranyl)- $\omega$ -hydroxypoly(oxy-1,2-ethandiyil)) is used as a solvent in parenteral products for intravenous or intramuscular injection of concentrations of up to 50% v/v; when administered parenterally to humans, quantities of glycofurool

20 should not exceed 0.07 ml per kg of body weight per day (Handbook of Pharmaceutical Excipients, Am. Pharmac. Assoc. and Pharm. Soc. of Gr. Br., 1994). However, heretofore, there has not been described a solution

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useful for the *in situ* formation of a biodegradable implant that is comprised of a biocompatible solvent being glycofurool and a biodegradable polymer being composed of lactic acid (LA) and/or glycolic acid (LG) units, e.g. PLGA, of which both polymer and solvent have been approved for 5 human administration, and which are compatible with each other, such that the polymer may be readily dissolved in the solvent at room temperature, without the need for other additives such as catalysts, additional solvents or the like; and which solution is useful for the *in situ* formation of a biodegradable implant for tissue or bone replacement, and 10 for the controlled release of drugs.

It is therefore an object of the invention to provide a composition comprising a polymer which is PLGA containing from 10-100 wt.% lactic acid (LA) units, preferably from 50-90 wt.% LA units, and a solvent which 15 is glycofurool for use in the formation of a biodegradable implant.

It is another object of the invention to provide a method which employs the composition of the invention in the *in situ* formation of a biodegradable implant.

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It is yet another object of the invention to provide biodegradable polymeric implants which can be as controlled-delivery devices for drugs or other biologically active agents.

5 It is another object the invention to provide implants the properties of which may be manipulated so as to enable the use of said implants with both hard and soft tissues.

It is still another object of the invention to provide biodegradable 10 polymeric implants comprising as polymer PLGA containing from 10-100 wt.% LA units, preferably 50-90 wt.% LA units, whenever prepared from the composition of the invention or by the method of the invention.

Other objects of the invention will become apparent as the description 15 proceeds.

#### Summary of the Invention

The present invention is based on the surprising finding that solutions of the polymer PLGA (poly(lactic-co-glycolic acid) containing from 10-100 20 wt.% LA units, preferably 50-90 wt.% LA units, in glycofurool ( $\alpha$ -(tetrahydrofuranyl)- $\omega$ -hydroxypoly(oxy-1,2-ethandiyl)), optionally compris-

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ing a drug or other biological agent, may be used to form biodegradable implants *in situ*.

The composition of the invention comprises a mixture of a polymer which  
5 is poly(lactic-co-glycolic acid) (PLGA) containing from 10-100 wt.% LA units, preferably 50-90 wt.% LA units, and a solvent which is  $\alpha$ -(tetrahydrofuranyl)- $\omega$ -hydroxypoly(oxy-1,2-ethandiyl) (glycofurool), optionally comprising a drug or other biologically active agent dissolved, dispersed or suspended in the mixture.

10

The method for *in situ* formation of a biodegradable implant, according to the invention, comprises the steps of:

- a - dissolving a polymer which is poly(lactic-co-glycolic acid) (PLGA) containing from 10-100 wt.% LA units, preferably 50-90 wt.% LA units, in  
15 a solvent which is  $\alpha$ -(tetrahydrofuranyl)- $\omega$ -hydroxypoly(oxy-1,2-ethandiyl) (glycofurool) to form a liquid;
- b - administering a suitable amount of said liquid to an animal body; and
- c - allowing said glycofurool to dissipate in said animal body,  
20 whereby to produce a solid implant composed of a PLGA polymeric matrix.

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Optionally, a drug or other biologically active molecule may be dissolved, dispersed or suspended in the liquid prior to, concurrent with or subsequent to said dissolution of said polymer.

5    The term "animal body" as used herein includes the human body.

The invention also comprises compositions comprising a mixture of  $\alpha$ -  
(tetrahydrofuryl)- $\omega$ -hydroxypoly(oxy-1,2-ethandiyil) (glycofurool) and a  
pharmaceutically acceptable, medically acceptable, or veterinarilly  
10 acceptable polymer which is soluble in glycofurool.

Other aspects and embodiments of the invention are set forth hereinbelow,  
or will readily arise from the following detailed description.

15    **Brief Description of the Drawings**

- Figs. 1A-1E show reproductions of scanning electronmicrographs  
of the surface of polymeric compositions formed by the method of the  
invention, at various periods of time after formation of each composition  
while each composition has been exposed to phosphate buffered solution  
20 (PBS);

- Fig. 2 shows graphically a release profile for the release of bovine  
serum albumin (BSA) from implants prepared from the solution of the

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invention as a function of PLGA (1:1 weight ratio LA to GA units, inherent viscosity 0.38 dl/g) concentration in the solution prior to formation of the solid;

- Fig. 3 shows graphically the release profile for the release of BSA from implants prepared from the solution of the invention as a function of inherent viscosity (average molecular weight) of PLGA (1:1 weight ratio LA to GA units);

- Fig. 4 shows graphically the release profile of BSA from implants prepared from the solution of the invention (PLGA of 1:1 weight ratio LA to GA units, inherent viscosity 0.38 dl/g) as a function of BSA concentration relative to the polymer prior to (and at the time of) formation of the solid;

- Fig. 5 shows graphically the release profile of BSA from implants prepared from the solution of the invention (PLGA of 3:1 weight ratio LA to GA units, inherent viscosity 0.59 dl/g) as a function of BSA concentration relative to the polymer prior to (and at the time of) formation of the solid;

-Fig. 6 shows graphically the release profile of human soluble p55 tumor necrosis factor receptor (sp55-R) from implants prepared from the formulation of the invention as a function of inherent viscosity (average molecular weight) of PLGA (1:1 weight ratio LA to GA units);

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-Fig. 7 shows graphically the release profile of sp55-R from implants prepared from the solution of the invention as a function of inherent viscosity (average molecular weight) of PLGA (3:1 weight ratio LA to GA units);

5 -Fig. 8 shows graphically the release profile of the total amount of protein (BSA alone, sp55-R alone, or BSA together with sp55-R) from implants prepared from the solution of the invention (PLGA with 1:1 weight ratio LA to GA units) as a function of the ratio of sp55-R to BSA (1:20 or 1:3600) and as a function of total protein loading (3 wt.% or 10  
10 wt.% relative to the weight of the polymer);

-Fig. 9 shows graphically the release profile of the total amount of protein (BSA alone, sp55-R alone, or BSA together with sp55-R) from implants prepared from the solution of the invention (PLGA with 3:1 weight ratio LA to GA units) as a function of the ratio of sp55-R to BSA  
15 (1:20 or 1:3600) and as a function of total protein loading (3 wt.% or 10 wt.% relative to the weight of the polymer);

-Fig. 10A shows the effect of the amount of protein loaded (3 wt.% and 10 wt.% with reference to polymer) and the nature of the protein (sp55-R alone or with BSA in a 1:20 weight ratio) on the release rate of  
20 protein from a 0.05 g formulation of 10 wt.% PLGA in glycofurool (1:1 weight ratio LA/GA units, 0.38 dl/g inherent viscosity) over a period of 100 hours; Fig 10B shows the same profile over a period of 35 days;

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-Fig. 11 shows the effect of polymer concentration (10 wt.% and 20 wt.% with reference to glycofurool, 1:1 weight ratio LA/GA units, inherent viscosity of 0.38 dl/g, 3 wt.% total protein relative to polymer) and the nature of the protein on the release rate over a period of over 30 days;

5 -Figs. 12A and 12B are similar to Figs. 10A and 10B, respectively, but for polymer with a 3:1 ratio LA/GA units, 0.59 dl/g inherent viscosity;

-Fig. 13 is similar to Fig. 11, but for polymer with a 3:1 ratio LA/GA units, 0.59 dl/g inherent viscosity;

10 -Fig. 14 shows the effect of injection of formulas of the invention on the weights of tumor-bearing mice;

-Fig. 15 shows the effect of injection of formulas of the invention on the mortality of healthy and tumor-bearing mice;

15 -Fig. 16 shows the rate of weight gain of tumor-bearing mice treated with compositions of the invention in comparison to the weight gain of healthy mice;

-Fig. 17 shows the rate of dissolution of implants formed according to the invention (10 or 20 wt.% polymer relative to glycofurool, 1:1 weight ratio LA/GA units) as determined by gel permeation chromatography (GPC); and

20 -Fig. 18 is similar to Fig. 17, but shows the results for implants containing a 3:1 weight ratio LA/GA units.

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**Detailed Description of the Invention**

Preferably, the average molecular weight of the glycofurool is between about 146.24 and 190.24. The density may be between about 1.07 and  
5 1.09 g/cm<sup>3</sup> at 20°C.

The polymer used in the invention may range in average molecular weight from about 2000 to about 100,000. The inherent viscosity may be between about 0.2 dl/g and 7.2 dl/g.

10

In the compositions of the invention, the weight ratio of glycofurool to polymer may be from about 99:1 to about 1:1. Preferably, the weight ratio of glycofurool to polymer is in the range of from about 9:1 to about 7:3.

15 For use in the method of the invention, PLGA may be present in the liquid composition prior to administration to the animal body in a weight ratio of about 1:99 to about 1:1 relative to glycofurool. Often, good results may be obtained with PLGA in weight ratio of between about 1:9 and 3:7 relative to glycofurool.

20

As used herein, the term "drug" or "biologically active molecule" includes any substance which is physiologically or pharmacologically active which

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acts locally or systemically in a body. Thus these molecules may be selected from among organic molecules, such as steroids; peptides or polypeptides; proteins, such as insulin, cytokines, their respective receptors and other therapeutic hormones and their receptors; oligo- or 5 polynucleotides; or other biologically active molecules. The amount of biologically active molecule present in the solution prior to placement in the animal may be up to an amount equal to the weight of the polymer present prior to placement in the animal. Preferably, when a drug or biologically active molecule is incorporated into the solution, the weight 10 ratio of the polymer to the biologically active molecule is between about 10000:1 and about 1:1

As compositions according to the invention, the liquid compositions used in carrying out the method of the invention are part of the invention.

15

The invention also comprises a method of treatment of an animal in need of a prosthetic implant, comprising the steps of:

a - dissolving poly(lactic-co-glycolic acid) copolymer (PLGA), containing from 10-100 wt.% lactic acid (LA) units, preferably from 50-90 20 wt.% LA units, in  $\alpha$ -(tetrahydrofuryl)- $\omega$ -hydroxypoly(oxy-1,2-ethandiyl) (glycofurool) to form a liquid;

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b - administering said liquid to an animal body; and  
c - allowing said glycofurool to dissipate to leave a solid  
implant in said animal body.

5 The invention also comprises a method of treating an animal, including  
man, in need of controlled or sustained release of a drug or other  
biologically active agent, comprising the steps of:

a - dissolving poly(lactic-co-glycolic acid) copolymer (PLGA)  
containing from 10-100 wt.% lactic acid (LA) units, preferably from 50-90  
10 wt.% LA units, in  $\alpha$ -(tetrahydrofuranyl)- $\omega$ -hydroxypoly(oxy-1,2-ethandiyil)  
(glycofurool) to form a liquid;

b - administering a suitable amount of said liquid to an  
animal in need of such administration; and

c - allowing said glycofurool to dissipate in said animal to  
15 leave a solid implant composed of a PLGA polymeric matrix;

wherein said liquid further comprises said drug or other biologically  
active agent, which has been dissolved, dispersed or suspended in said  
glycofurool prior to, concurrently with, or subsequent to said dissolution of  
PLGA.

20

The invention also comprises biodegradable implants, whenever prepared  
by the method of the invention or from a solution of the invention.

As stated, biodegradable implants can serve several functions, including use as a prosthetic or orthodontic implant. The present invention may thus be used where tissue regeneration is desired, e.g. where a growth has  
5 been removed from a body and the resulting space requires filling as the tissue grows back, or where ingrowth of bone tissue into a space is necessary. The present invention may also be used for the sustained release of a drug or other biologically active agent. In the context of the invention, a wide variety of drugs and other biological agents, such as  
10 peptides (e.g. peptide hormones), proteins (e.g. growth factors, interferons, cytokines and cytokine-binding proteins), oligo- or polynucleotides, and organic compounds (e.g captopril, steroids, prostaglandins and the like), and other molecules with biological function (e.g narcotic antagonists, anti-arrhythmics, anti-cancer agents, hormone antagonists, contraceptive  
15 agents, and anti-inflammatory agents) may be incorporated into the composition; and thus the invention may be employed in the treatment of a variety of diseases or conditions. For example, a controlled-release device containing insulin, prepared from the composition of the invention, can be used in the treatment of diabetes. Other drugs which at present  
20 may be delivered by controlled-release systems--such as those systems using microspheres--e.g. leutinizing hormone releasing hormone (LHRH),

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bromocriptine, or contraceptives, may also be used in context of the present invention.

According to the invention, in those aspects of the invention which require administration to an animal body, the mode of administration of the composition of the invention may be any suitable mode of administration. It will be understood that since one of the advantages of the present invention is the ability to place an implant in an animal body without resorting to surgery, a preferred mode of administration is injection, e.g. through a syringe and needle. It will also therefore be understood that preferred compositions of the invention are those which are injectable, and that preferred methods of the invention are those which employ injection as the means of administration of the compounds of the invention.

15 **Examples**

The foregoing characteristics and advantages of the invention, such as the use of components which have independently been approved for use by the US Food and Drug Administration (FDA) and other national health authorities, will be better understood through the following illustrative 20 and non-limitative examples.

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Example 1

Formation and Degradation of Polymeric Composition

Five samples of implants (listed in Table 1) were prepared as follows:

Poly(lactic-co-glycolic acid) copolymer (PLGA) (0.6 or 0.8 g) was dissolved in

5 glycofurol (3.4 g or 3.2 g, respectively) at room temperature. A sample of each liquid (4 g) in drops of 250  $\mu$ l was then poured into water (10 ml phosphate buffered saline solution, PBS, pH 7.4 at 37°C on shaker bath), and immediately the polymer solidified to form a polymeric composition.

The PBS medium was changed daily and the composition surface

10 photographed under a scanning electron microscope at intervals of 4-7 days. As can be seen from Figs. 1A-1E, which shows the surface of each sample composition from Table 1 at the times after formation indicated, the compositions slowly decay in the aqueous solvent.

15 **Table 1**

Sample #	Weight ratio LA/GA units in PLGA	Inherent viscosity, dl/g	wt.% PLGA relative to total wt. of sol'n	Appears as Fig. no.
1	50/50	0.38	15	1A
2	50/50	0.38	20	1B
3	50/50	0.47	15	1C
4	75/25	0.59	20	1D
5	75/25	0.59	15	1E

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Example 2

Polymeric composition containing protein (BSA) and effects of  
composition on release characteristics

5 This example shows that the invention may be used to prepare a controlled-release device, e.g. for medical use, as well as the effects of polymer concentration on the characteristics of release. Three samples as shown in Table 2 were prepared by dissolving PLGA (50/50 LA/GA weight ratio) in glycofurool as in Example 1, but powdered bovine serum albumin (BSA) was dispersed into the PLGA solution using vortex and probe sonication at 50W for 30 s, while the solution was kept on ice. In vitro release profiles were obtained by injecting 0.25 ml of the solution containing BSA into 10 ml of PBS to obtain solid matrices containing BSA. Samples were continuously shaken at 37°C. Release of BSA was quantitated by measuring the absorbence of the PBS at 280 nm. Fig. 2 shows the release profile of BSA as a function of the PLGA concentration in glycofurool.

10

15

**Table 2**

Sample no.	PLGA, g	Glycofurool, g	BSA, g	shorthand
6	0.4	3.6	0.012	10% PLGA 50/50, 3% BSA loading
7	0.6	3.4	0.018	15% PLGA 50/50, 3% BSA loading
8	0.8	3.2	0.024	20% PLGA 50/50, 3% BSA loading

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Example 3

Effects of inherent viscosity on release kinetics of BSA

Two samples analogous to sample 7 were prepared, but using PLGA with inherent viscosity of 0.38 dl/g or 0.47 dl/g. Fig. 3 shows the release  
5 kinetics for the matrices so obtained.

Example 4

Effects of BSA loading on release kinetics

Samples analogous to sample 7 were prepared using PLGA of inherent  
10 viscosity 0.38 dl/g, but the amount of BSA incorporated was varied to be 2,  
3, 10, 20 or 30 wt.% relative to the PLGA (2, 3, 10, 20 and 30% loading,  
respectively) prior to injection. The release profiles of the matrices so  
obtained can be seen in Fig. 4.

15

Example 5

Effects of ratio of LA/GA unit ratio on release kinetics

Samples analogous to sample 6 were prepared using PLGA containing an  
LA/GA weight ratio of 3:1 (inherent viscosity 0.59 dl/g), with BSA loading  
varied to be 1.5, 3, 10, 20 or 30%. The release profiles of the matrices so  
20 obtained can be seen in Fig. 5.

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Example 6

Effects of inherent viscosity on release kinetics of sp55-R

Four samples analogous to sample 6 were prepared, using PLGA (1:1 LA/GA weight ratio) with inherent viscosity of 0.24 dl/g, 0.38 dl/g, 0.47 5 dl/g and 0.55 dl/g, and using a powder mixture of sp55-R and BSA (3 wt.% total protein relative to polymer) in a 1:20 weight ratio. Fig. 6 shows the release kinetics for the matrices so obtained.

Example 7

10      Effects of inherent viscosity and ratio of LA/GA unit ratio on  
release kinetics of sp55-R

Four samples analogous to sample 6 were prepared, using PLGA (1:1 or 3:1 LA/GA weight ratio) with inherent viscosity of 0.24 dl/g or 0.55 dl/g, and using a powder mixture of sp55-R and BSA (3 wt.% total protein 15 relative to polymer) in a 1:20 weight ratio. Fig. 7 shows the release kinetics for the matrices so obtained.

Example 8

Release kinetics of mixtures of BSA and sp55-R

20      Samples analogous to sample 6, containing either BSA alone, sp55-R alone, or a combination of BSA and sp55-R in a weight ratio of 20:1 or 3600:1, and loaded with 3 wt.% or 10 wt.% total protein relative to the

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weight of the polymer (PLGA with a 1:1 weight ratio of LA/GA), were prepared. Fig. 8 shows the release kinetics of protein from these matrices.

**Example 9**

5       **Release kinetics of mixtures of BSA and sp55-R**

Samples analogous to sample 6, containing either BSA alone, sp55-R alone, or a combination of BSA and sp55-R in a weight ratio of 20:1, and loaded with 3 wt.% or 10 wt.% total protein relative to the weight of the polymer (PLGA with a 3:1 weight ratio of LA/GA), were prepared. Fig. 9  
10 shows the release kinetics of protein from these matrices.

**Example 10**

**Release of sp55-R from compositions in mice and protection of mice from chronic exposure to Tumor Necrosis Factor (TNF)**

15      Balb/c and Balb/c nude female mice, aged 8-9 weeks and weighing 20-21 g each, were injected with formulations of the invention containing sp55-R alone or in combination with BSA (or with formulations devoid of protein), and the release kinetics of sp55-R (and BSA) from the implant which resulted were measured; when the formulations contained sp55-R, the  
20 receptor was present in an amount of about 7-20 µg per mouse. As shown in the Figs. 10-14, the formulations used contained 10 wt.% (and in Figs. 11 and 13, 20 wt.% as well) PLGA (with a 1:1 weight ratio LA/GA units in

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Figs. 10 and 11, and a 3:1 LA/GA weight ratio in Figs. 12 and 13) in glycofurool, 3 wt.% to 10 wt.% total protein loading vs. polymer when containing protein, and BSA and sp55-R in a weight ratio of 20:1 when a mixture of BSA and sp55-R was used.

5

Serum samples were collected periodically after injections by tail bleeding and via the eye artery, and allowed to clot. The sp55-R level in these serum samples was determined by a 2-site capture enzyme-linked immunosorbent assay (ELISA). Figs. 10A and 12A show that after about 10 36 hours, the rate of release of protein remained stable for the next several days, and Figs. 10B, 11, 12B and 13 show that this rate of release remained stable over a period of several weeks. Figs. 11 and 13 show that the concentration of polymer used (10 wt.% vs. 20 wt.%) had negligible effect on the release profile.

15

Some of the mice used to determine the protein release profiles were also used to determine the effectiveness of the formulation of the invention as a means for mitigating the effects of chronic exposure to TNF. Balb/c nude female mice, aged 8-9 weeks and weighing 20-21 grams, were inoculated 20 subcutaneously in the flank area with Chinese hamster ovary (CHO) cells transfected with the TNF gene and expressing TNF. Injection of the

-24-

PLGA formulation into these mice was performed 5 days after tumor cell inoculation.

It is known that nude mice bearing TNF-producing tumors exhibit severe  
5 cachexia, leading to death (Oliff et al., Cell 50:555-563 (1987)). Injection  
of TNF-binding proteins, such as TNF-binding antibodies or TNF  
receptors, can temporarily mitigate these effects, but the injected proteins  
are rapidly cleared from the body, making direct injection of TNF-binding  
compounds a less-than-ideal means for mitigating the effects of chronic  
10 TNF exposure.

In accordance with the invention, however, injection of PLGA formulations  
containing sp55-R into such tumor-bearing mice (about 7 to 20 µg  
receptor/mouse) prevented body weight loss (body weight increased  
15 naturally). This is illustrated in Fig. 14, which shows that over a period of  
50 days, the weight of tumor-bearing mice treated with a formulation of  
the invention (10 wt.% PLGA (3:1 LA:GA ratio), 0.59 dl/g in glycofurool, 3  
wt.% loading of sp55-R with BSA (7.1 µg sp55-R)) ( $\Delta$ ) increased at a  
similar rate to that of healthy Balb/c nude mice ( $\bullet$ ); whereas untreated  
20 tumor-bearing mice ( $O$ ) and tumor-bearing mice injected with a  
formulation of the invention containing only polymer ( $\blacksquare$ ) lost about 25%  
body weight over the course of the experiment. In Fig. 16, it can be seen

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that tumor-bearing mice injected with TNF-loaded implants containing 20 wt.% polymer relative to glycofurol ( $\blacktriangle$ ) gained weight at a rate comparable to the rate of weight gain of tumor-bearing mice injected with TNF-loaded formulation containing 10 wt.% polymer relative to 5 glycofurol ( $\Delta$ ).

Similarly, Fig. 15 shows the survival rate for various groups of tumor-bearing mice. Only those tumor-bearing mice which were untreated (O) or treated with empty PLGA formulation (■) died over the course of the 10 experiment; tumor-bearing mice injected with formulations containing sp55-R( $\Delta$  and  $\blacktriangle$ , 10% wt.% polymer and 20 wt.% polymer, respectively, relative to glycofurol), healthy mice injected with empty formulation (□), and healthy mice which did not receive injections (●), all survived the test period.

15

#### Example 11

##### Degradation of Implants in PBS

Implants were prepared *in vitro* by direct injection into PBS (pH 7.4) of 200 mg of formulations containing 10 or 20 wt.% polymer (inherent 20 viscosity 0.38, 0.24, or 0.55 dl/g) relative to glycofurol, with a 1:1 ratio of LA to GA units. The implants thus formed were maintained at 37°C on a shaker bath, and then dried under vacuum at various intervals for use in

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gel permeation chromatography (GPC). The average molecular weight of the implants was determined using a Waters 510 pump with a Waters RI-410 refractive index detector. Tetrahydrofuran (THF) was used for the mobile phase, at a flow rate of 1 ml/min and temperature of 30°C.

5      Implants were dissolved in THF (0.25 wt.% polymer sample in THF), filtered and then injected as a 20 µl sample into a set of four µ-Styragel columns (Waters) with nominal pore sizes of 10<sup>-5</sup>, 10<sup>-6</sup>, 10<sup>-7</sup>, and 10<sup>-8</sup> m. Average molecular weights were calculated using a series of polystyrene standards which ranged from 162 to 194000 molecular weight (Mw). The  
10     results are shown in Fig. 17.

#### Example 12

##### Degradation of Implants in PBS

The procedure of Example 11 was followed, but the implants were  
15     prepared from formulations containing PLGA (inherent viscosities of 0.24 and 0.59 dl/g) containing a 3:1 ratio of LA to GA units. The results are shown in Fig. 18.

20     The foregoing examples are given for illustrative purposes only. Within the scope of the invention, many modifications on the foregoing can be made. For example, small organic molecules, peptides, polypeptides, oligo- or polynucleotides, in various concentrations, can be incorporated

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into the solution; the average molecular weight of the polymer can be varied; the relative amounts of lactic acid and glycolic acid units can be varied; and the relative amounts of polymer and solvent can be varied, all without exceeding the scope of the invention.

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CLAIMS

1. A composition comprising a mixture of  
a polymer which is poly(lactic-co-glycolic acid) copolymer (PLGA)  
containing between about 10 and 100 wt.% lactic acid (LA) units,  
5 preferably between about 50 and 90 wt.% LA units, and  
 $\alpha$ -(tetrahydrofuryl)- $\omega$ -hydroxypoly(oxy-1,2-ethandiyl) (glycofurool).
2. A composition according to claim 1, said composition being a  
solution comprising a polymer which is poly(lactic-co-glycolic acid)  
10 copolymer (PLGA) containing between about 10 and 100 wt.% lactic acid  
(LA) units, preferably between about 50 and 90 wt.% LA units, said  
polymer being dissolved in  $\alpha$ -(tetrahydrofuryl)- $\omega$ -hydroxypoly(oxy-1,2-  
ethandiyl) (glycofurool).
- 15 3. A composition according to claim 1 or 2, further comprising a  
biologically active molecule from the group comprising proteins,  
polypeptides, peptides, organic molecules, oligo- or polynucleotides, or  
other biologically active molecules.
- 20 4. A composition according to claim 1 or 2, wherein the polymer has an  
average molecular weight between about 2,000 and about 100,000.

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5. A composition according to claim 1 or 2, wherein the polymer has an intrinsic viscosity between about 0.2 and about 7.0 dl/g.
- 5 6. A composition according to claim 1 or 2, wherein the weight ratio of glycofurool to polymer is between about 99:1 and about 1:1.
7. A composition according to claim 6, wherein the weight ratio of glycofurool to polymer is between about 9:1 and about 7:3.
- 10 8. A composition according to claim 3, wherein the weight ratio of the polymer to the biologically active molecule is between about 10000:1 and about 1:1.
- 15 9. Use of a composition according to any one of claims 1 to 8 in the preparation of a prosthetic or orthodontic implant.
10. A composition according to any one of claims 1 to 8 for use in the preparation of a prosthetic or orthodontic implant.

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11. Use of a composition according to any one of claims 1 to 8 in the preparation of a device for the controlled and/or sustained release of one or more drugs or other biologically active molecules.
- 5    12. A composition according to any one of claims 1 to 8 for use in the preparation of a device for the controlled and/or sustained release of one or more drugs or other biologically active molecules.
13. A method for producing a solid implant *in situ* in an animal body, including man, comprising the steps of:
  - a - dissolving a polymer which is poly(lactic-co-glycolic acid) copolymer (PLGA) containing from about 10-100 wt.% lactic acid (LA) units, preferably from about 50-90% LA units, in  $\alpha$ -(tetrahydrofuryl)- $\omega$ -hydroxypoly(oxy-1,2-ethandiyl) (glycofurool) to form a liquid;
  - 15    b - administering a suitable amount of said liquid to an animal, including man, in need thereof; and
  - c - allowing said glycofurool to dissipate in said animal to leave a solid implant composed of a PLGA polymeric matrix.
- 20    14. The method of claim 13, wherein said composition in liquid form further comprises a biologically active molecule selected from the group

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comprising proteins, polypeptides, peptides, organic molecules, oligo- or polynucleotides, or other biologically active molecules.

15. The method of claim 13, wherein said administering of said liquid is  
5 accomplished by injection through a needle.

16. The method of claim 13, wherein the weight ratio of glycofurool to polymer prior to administration is between about 99:1 and about 1:1.

10 17. The method of claim 13, wherein the polymer has an average molecular weight between about 2,000 and about 100,000.

18. The method of claim 13, wherein the polymer has an inherent viscosity between about 0.2 and about 7.2 dl/g.

15

19. The method of claim 14, wherein the weight ratio of said polymer to said biologically active molecule prior to administration is between about 10000:1 and about 1:1.

20 20. A method of treating an animal, including man, in need of a prosthetic or orthodontic implant comprising the steps of:

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a - dissolving a polymer which is poly(lactic-co-glycolic acid) copolymer (PLGA) containing between about 10-100 wt.% lactic acid (LA) units, preferably between about 50-90 wt.% LA units, in  $\alpha$ -(tetrahydrofuranyl)- $\omega$ -hydroxypoly(oxy-1,2-ethandiyl) (glycofurool) to form  
5 a liquid;

b - administering said liquid to an animal body; and

c - allowing said glycofurool to dissipate to leave a solid implant in said animal body.

10 21. A method of treating an animal, including man, in need of controlled or sustained release of a drug or other biologically active agent, comprising the steps of:

a - dissolving a polymer which is poly(lactic-co-glycolic acid) copolymer (PLGA) containing between about 10-100 wt.% lactic acid (LA) units, preferably between about 50-90 wt.% LA units, and dissolving, dispersing or suspending said drug or other biologically active agent, in  $\alpha$ -(tetrahydrofuranyl)- $\omega$ -hydroxypoly(oxy-1,2-ethandiyl) (glycofurool) to form a liquid, wherein said dissolving, dispersing or suspending of said drug or other biologically active agent may take place previously, concurrently, or  
15 subsequently to said dissolving of said polymer;

b - administering said liquid to an animal body; and

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c - allowing said glycofurool to dissipate to leave a solid, controlled-release device in said animal body.

22. A solid implant, whenever prepared by the method of claim 13.

5

23. A solid implant, whenever prepared from a solution according to  
claim 1.

24. A method according to claim 21, whereby to produce a solid, controlled  
10 release device which releases said drug or which releases said drug or  
biologically active agent by diffusion, erosion or a combination of diffusion  
and erosion as said device degrades in said animal body.

25. The method of claim 24, wherein said implant is formed in a  
15 periodontal pocket in said body.

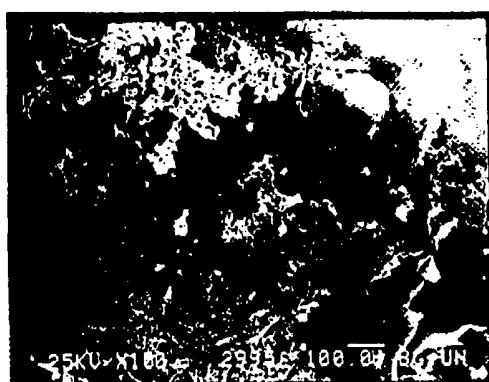
26. A solid, controlled release device whenever prepared by the method of  
claim 21.

20 27. A composition comprising a mixture of  $\alpha$ -(tetrahydrofuryl)- $\omega$ -  
hydroxypoly(oxy-1,2-ethandiyl) (glycofurool) and a pharmaceutically  
acceptable, medically acceptable or veterinarilly acceptable polymer which

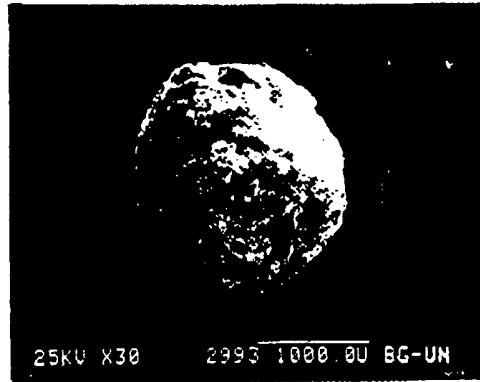
-34-

is soluble in  $\alpha$ -(tetrahydrofuryl)- $\omega$ -hydroxypoly(oxy-1,2-ethandiyl) (glycofurool).

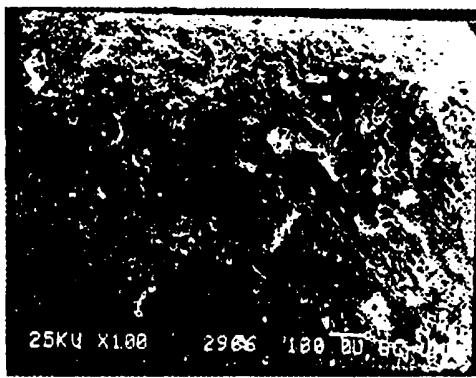
1/24



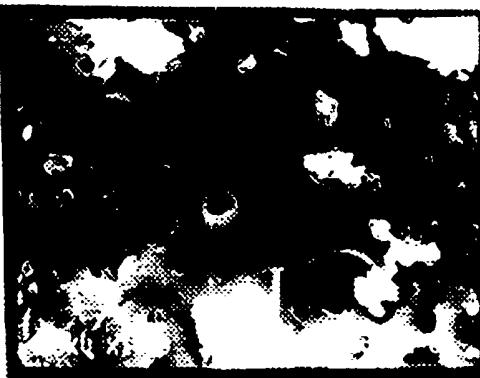
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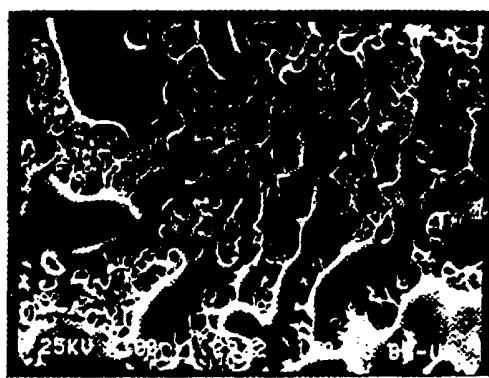
(b) After 16 hours



(c) After 14 days



(d) After 14 days



(e) After 27 days



(f) After 27 days

Fig. 1A

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(a) After 16 hours



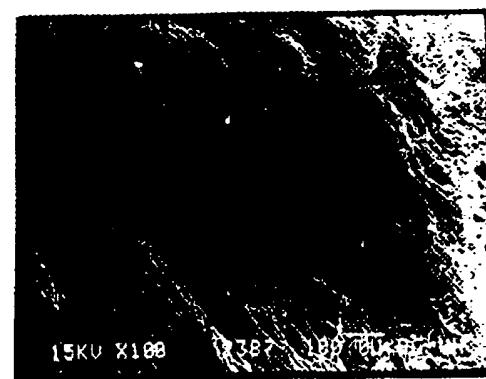
(b) After 16 hours



(c) After 14 days



(d) After 27 days



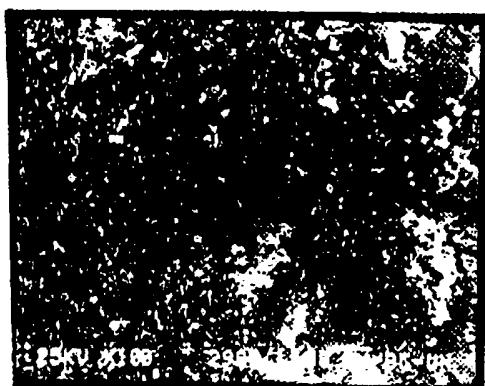
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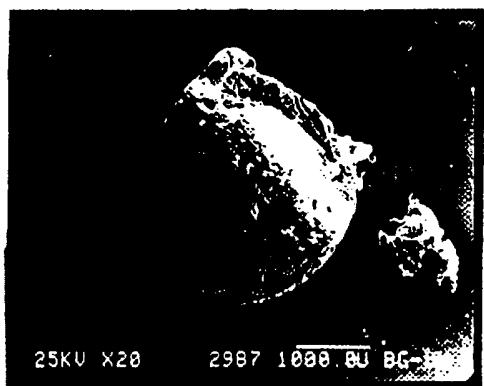
(f) After 32 days

Fig. 1B

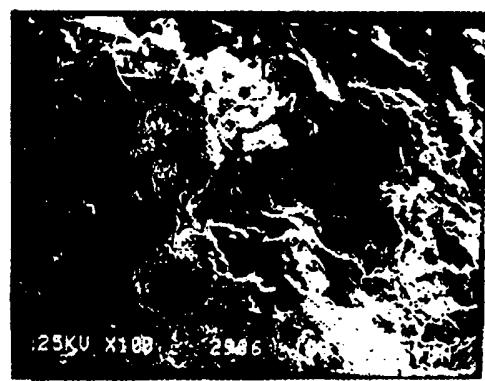
3/24



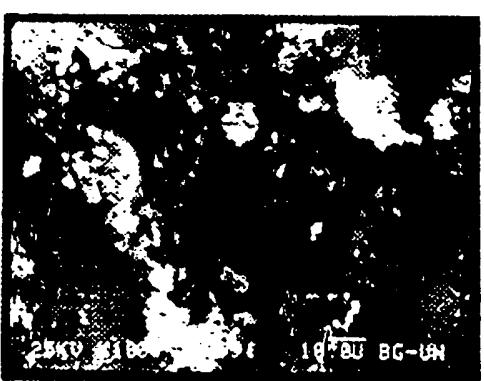
(a) After 16 hours



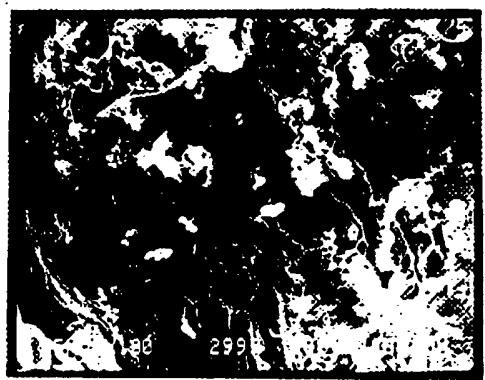
(b) After 16 hours



(c) After 16 hours



(d) After 14 days



(e) After 14 days



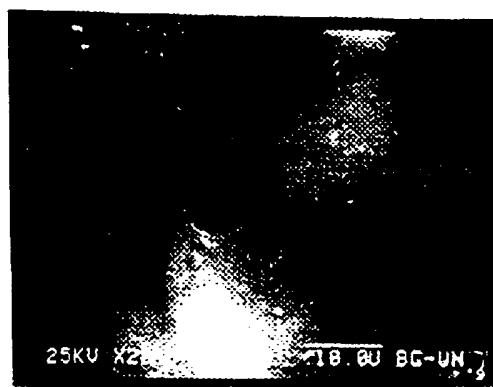
(f) After 27 days

Fig. 1C

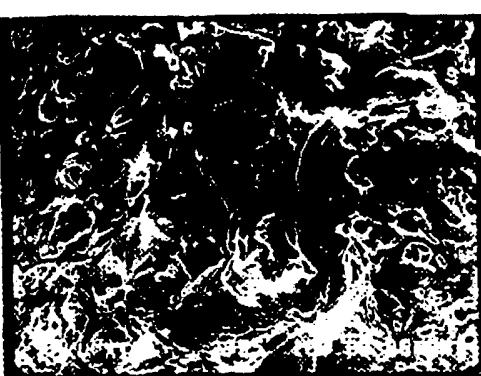
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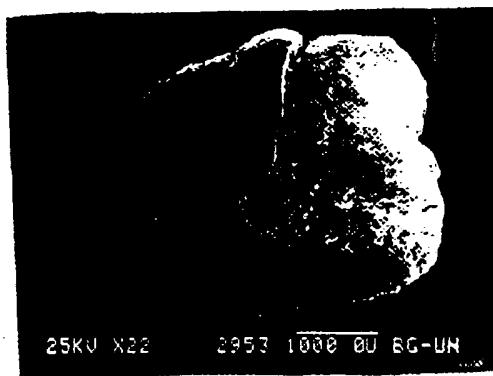
(a) After 24 hours



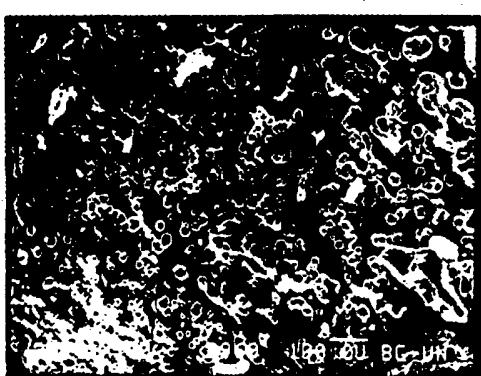
(b) After 24 hours



(c) After 21 days



(d) After 21 days



(e) After 36 days



(f) After 36 days

Fig. 1D

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(a) After 24 hours



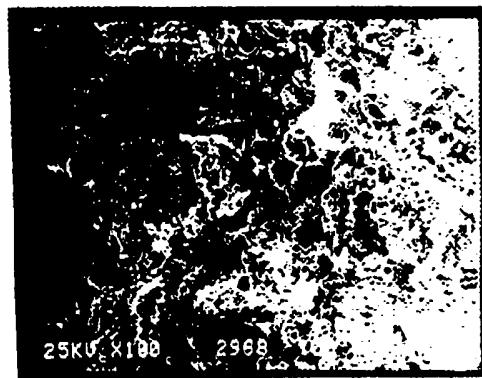
(b) After 24 hours



(c) After 21 days



(d) After 21 days



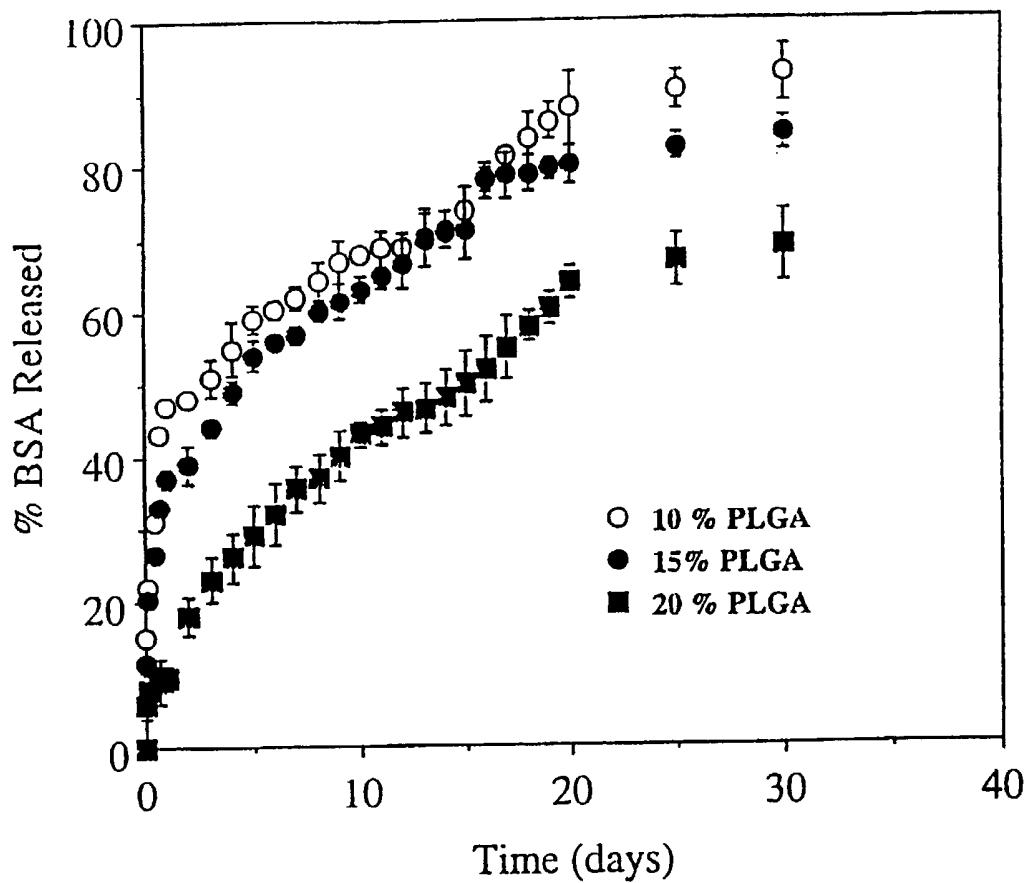
(e) After 32 days



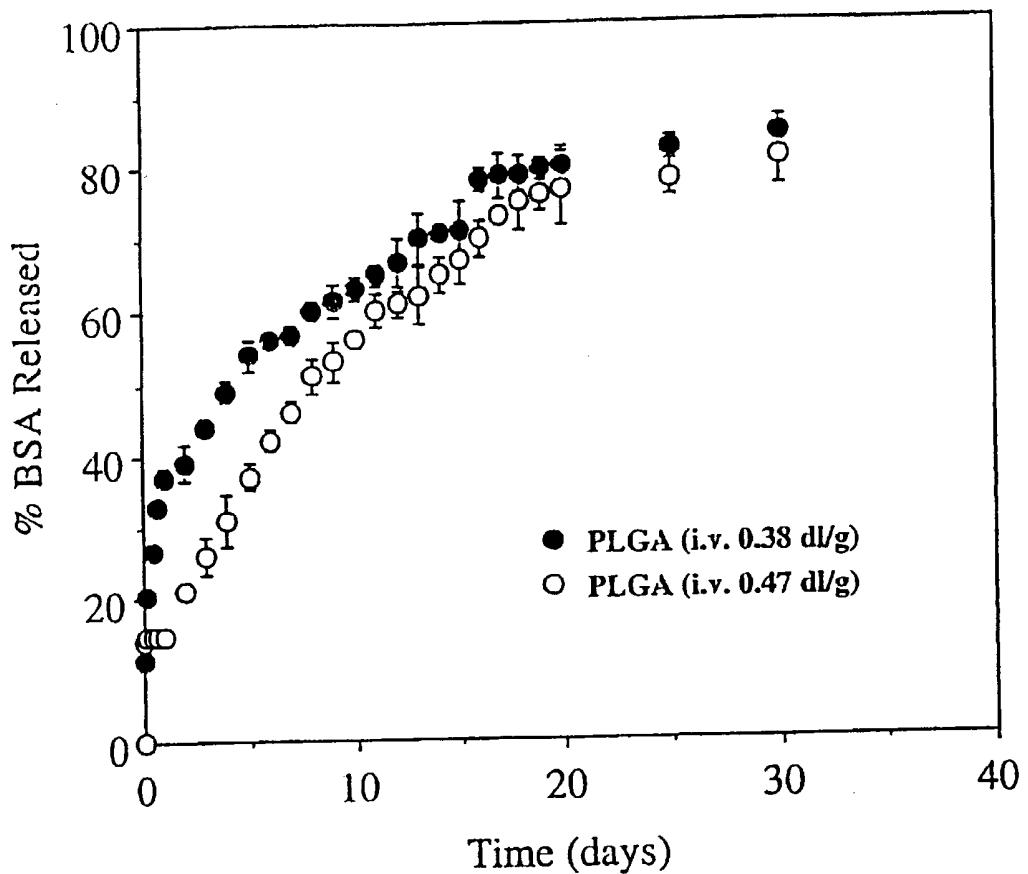
(f) After 32 days

Fig. 1E

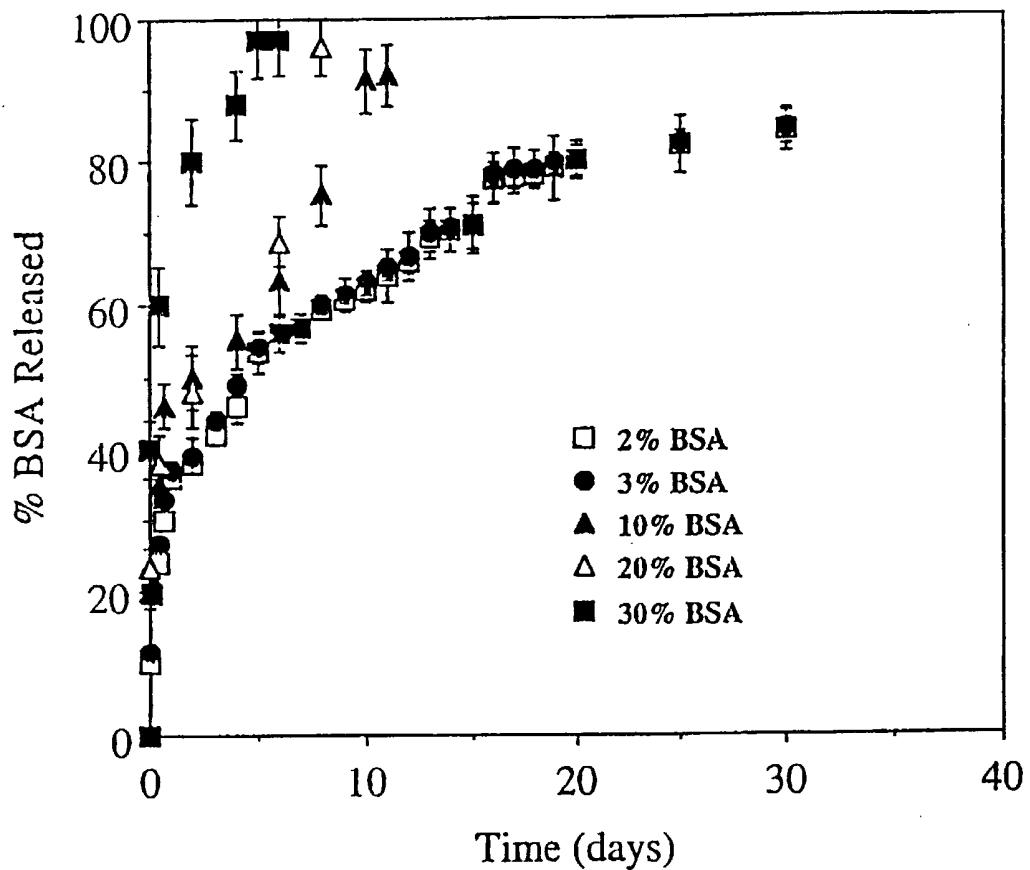
6/24

**Figure 2.**

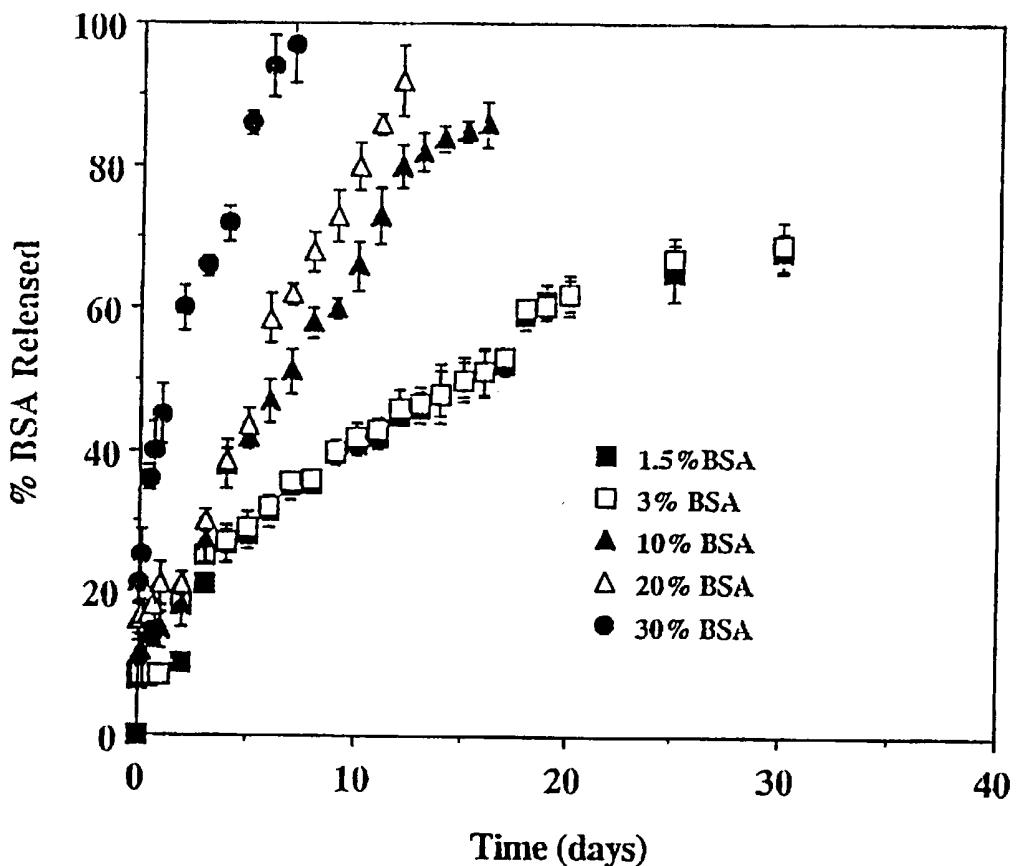
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**Figure 3.**

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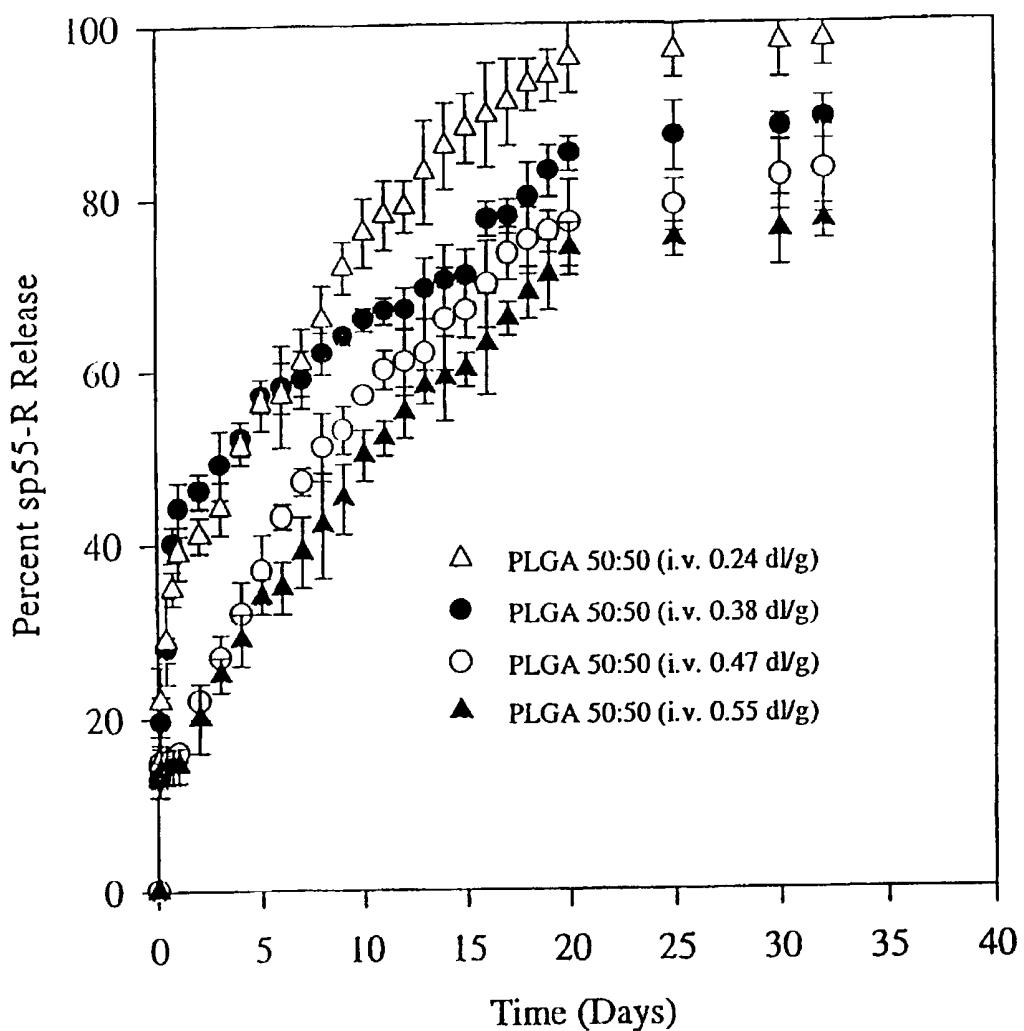
**Figure 4.**

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**Figure 5.**

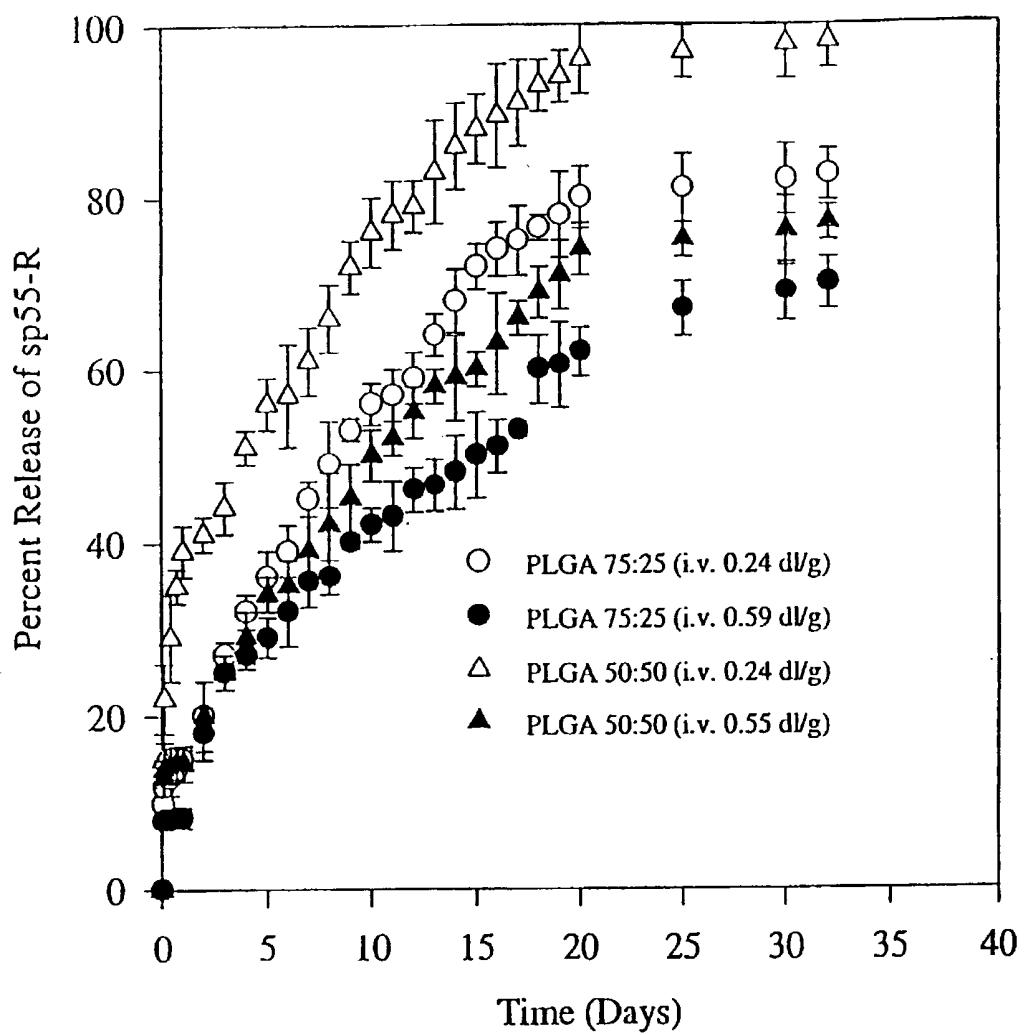
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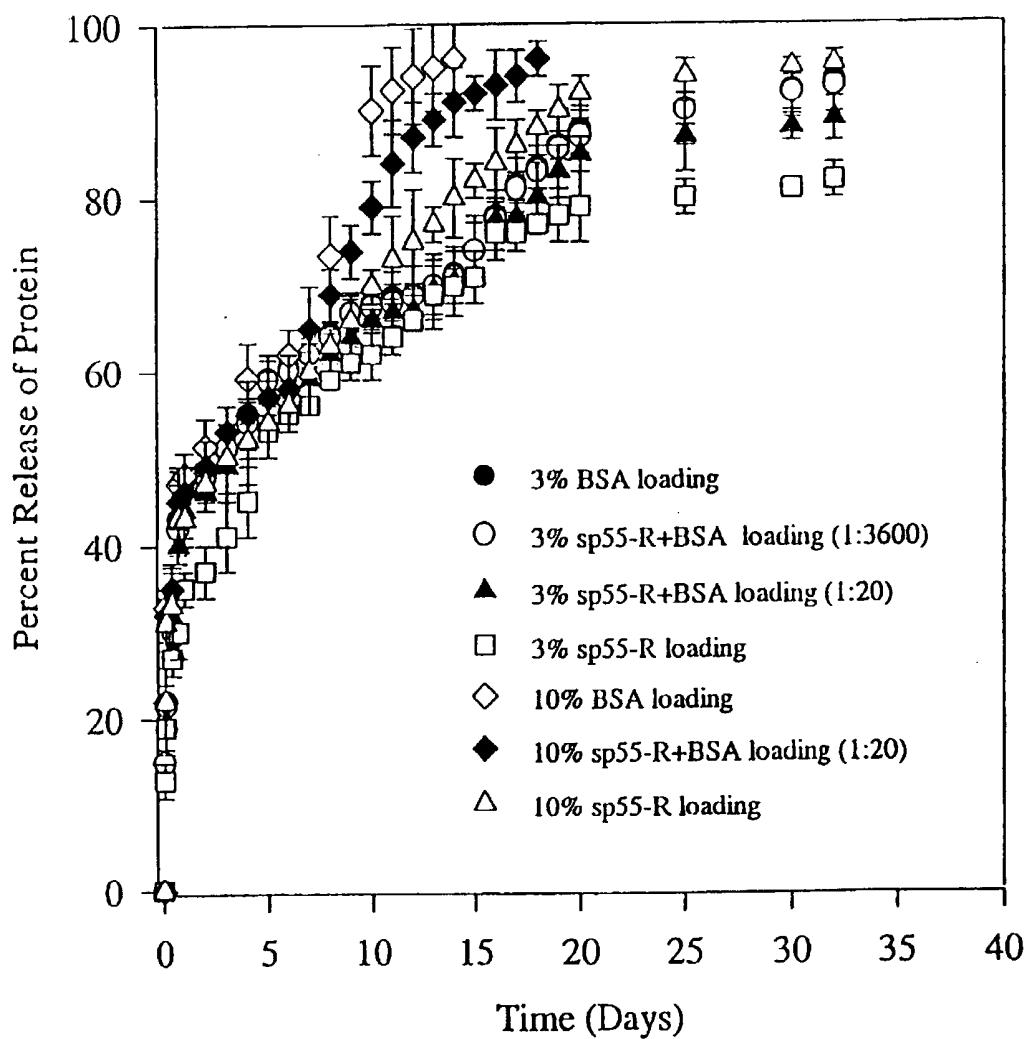
Fig. 6



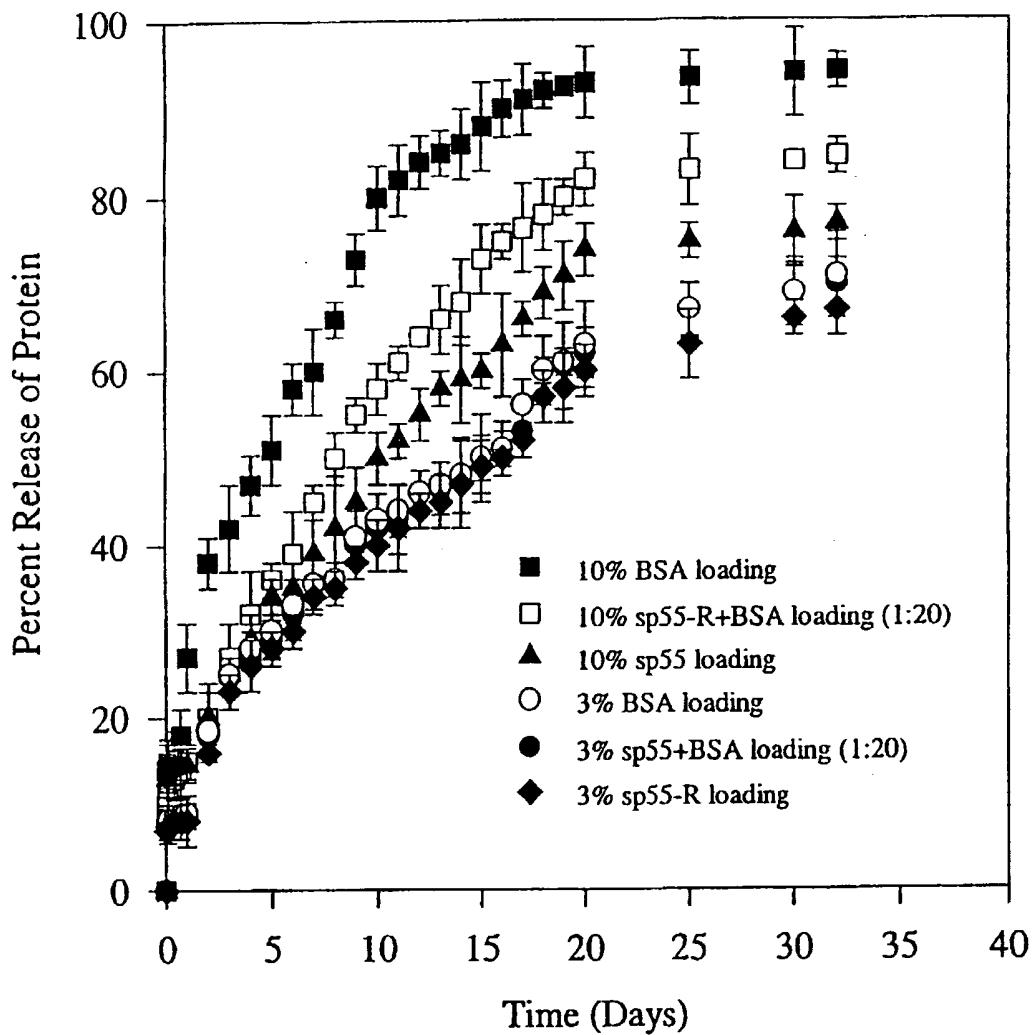
11/24

Fig. 7

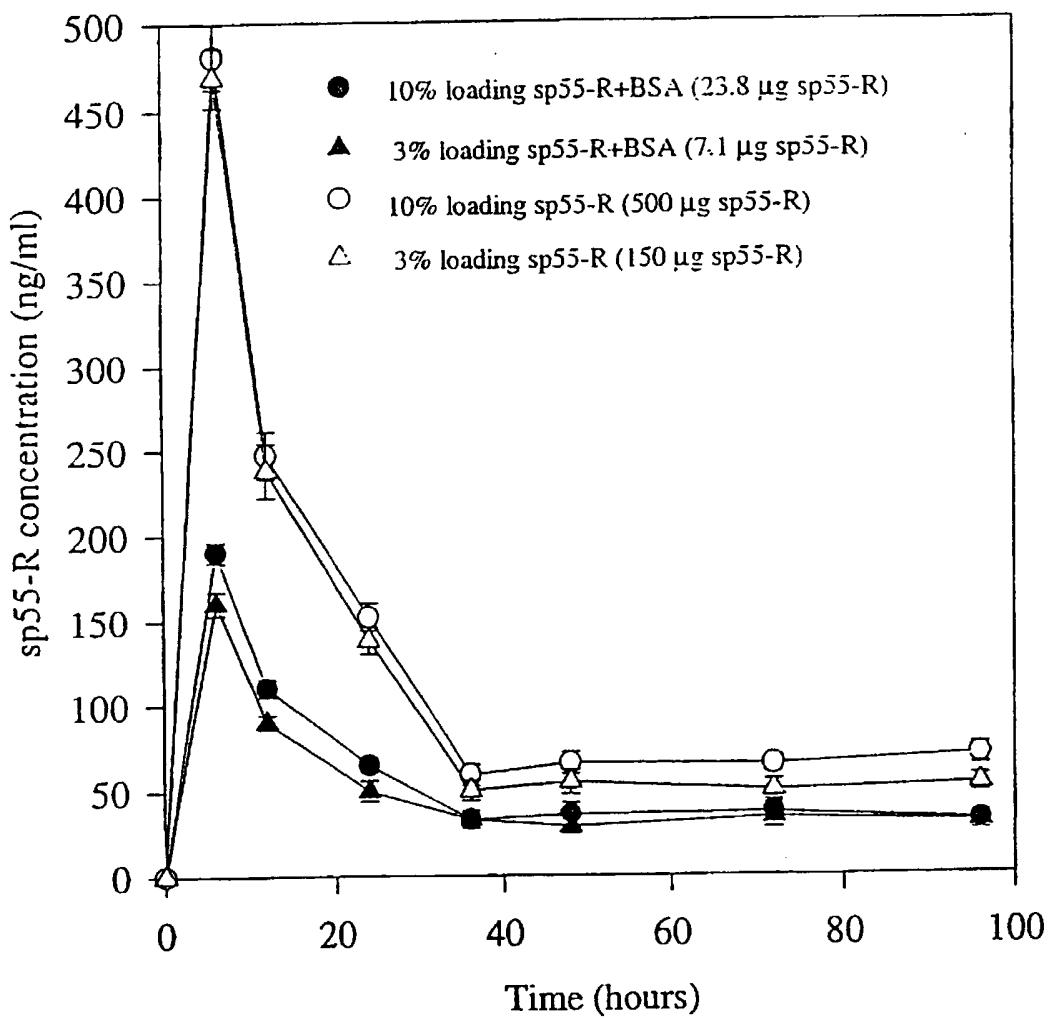


**Fig. 8**

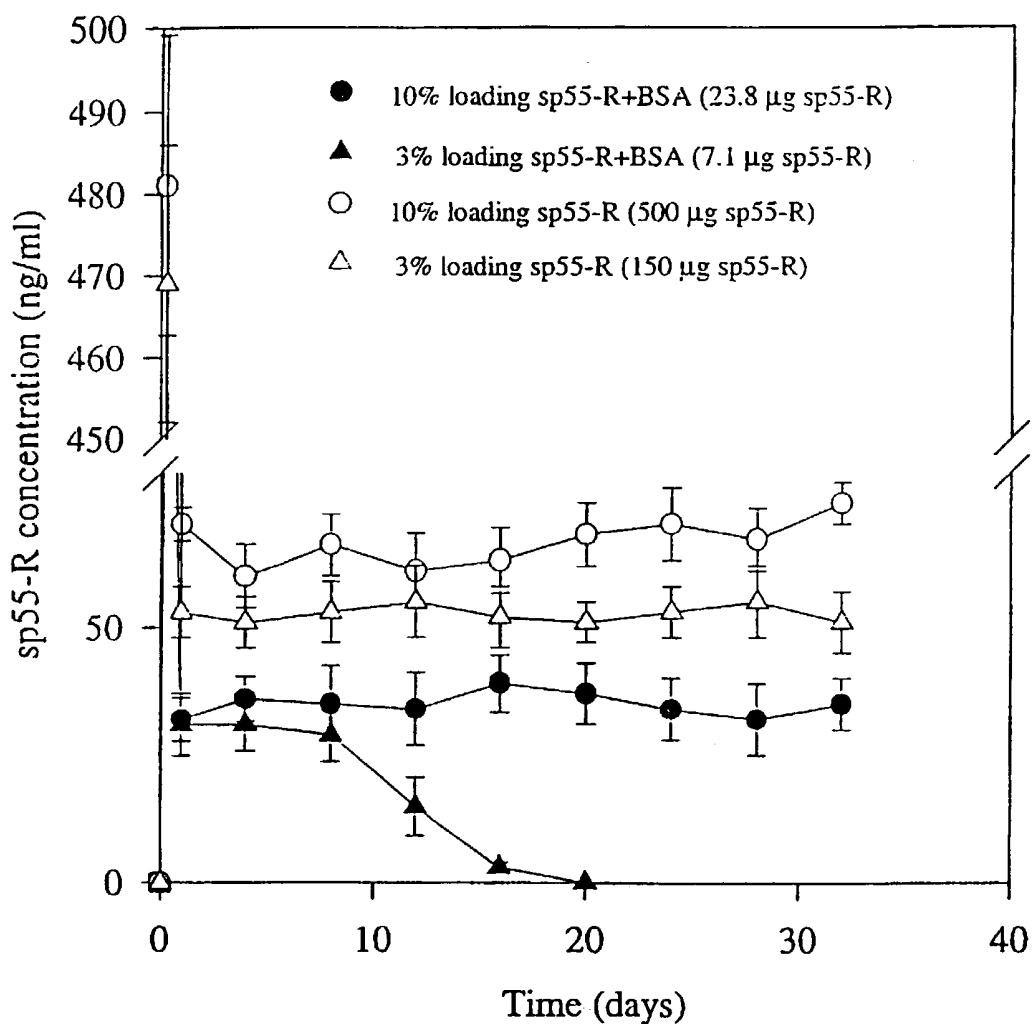
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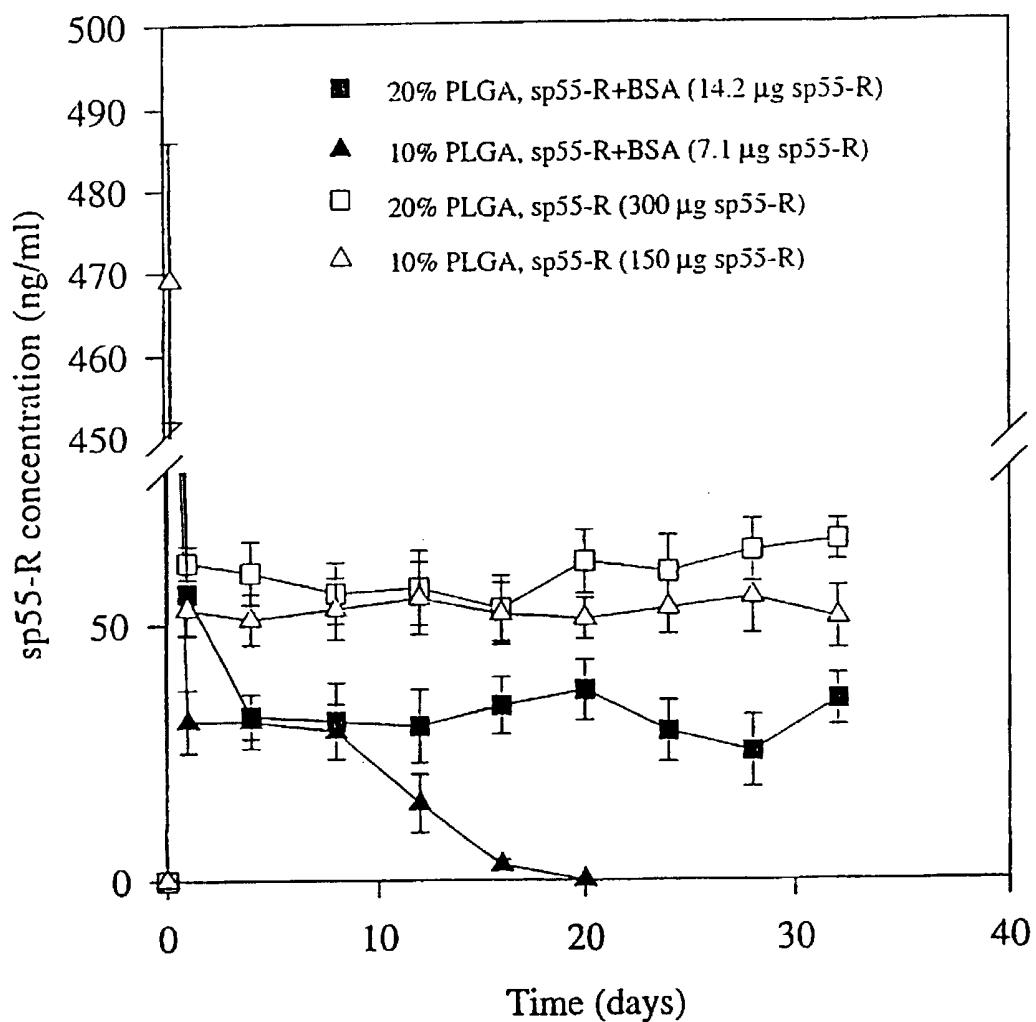
**Fig. 9**

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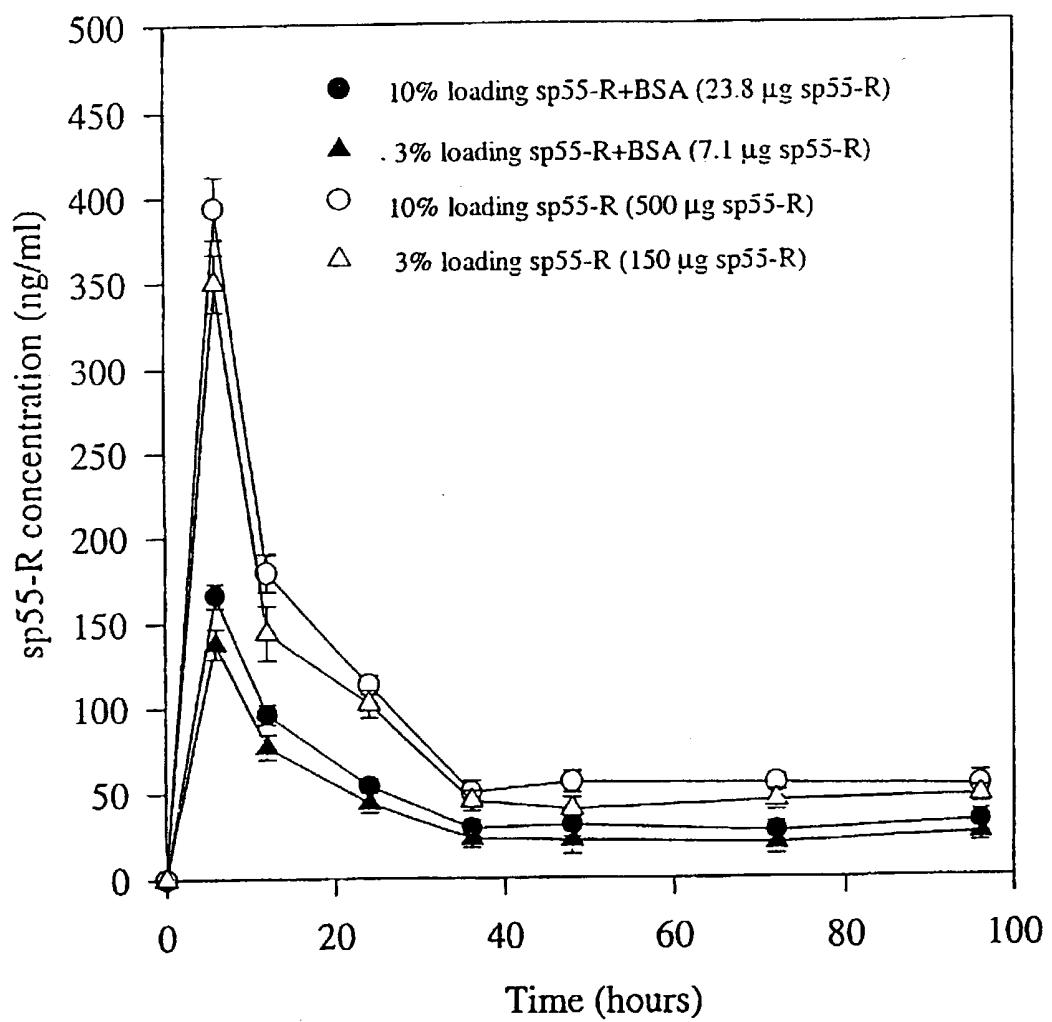
**Fig. 10A**

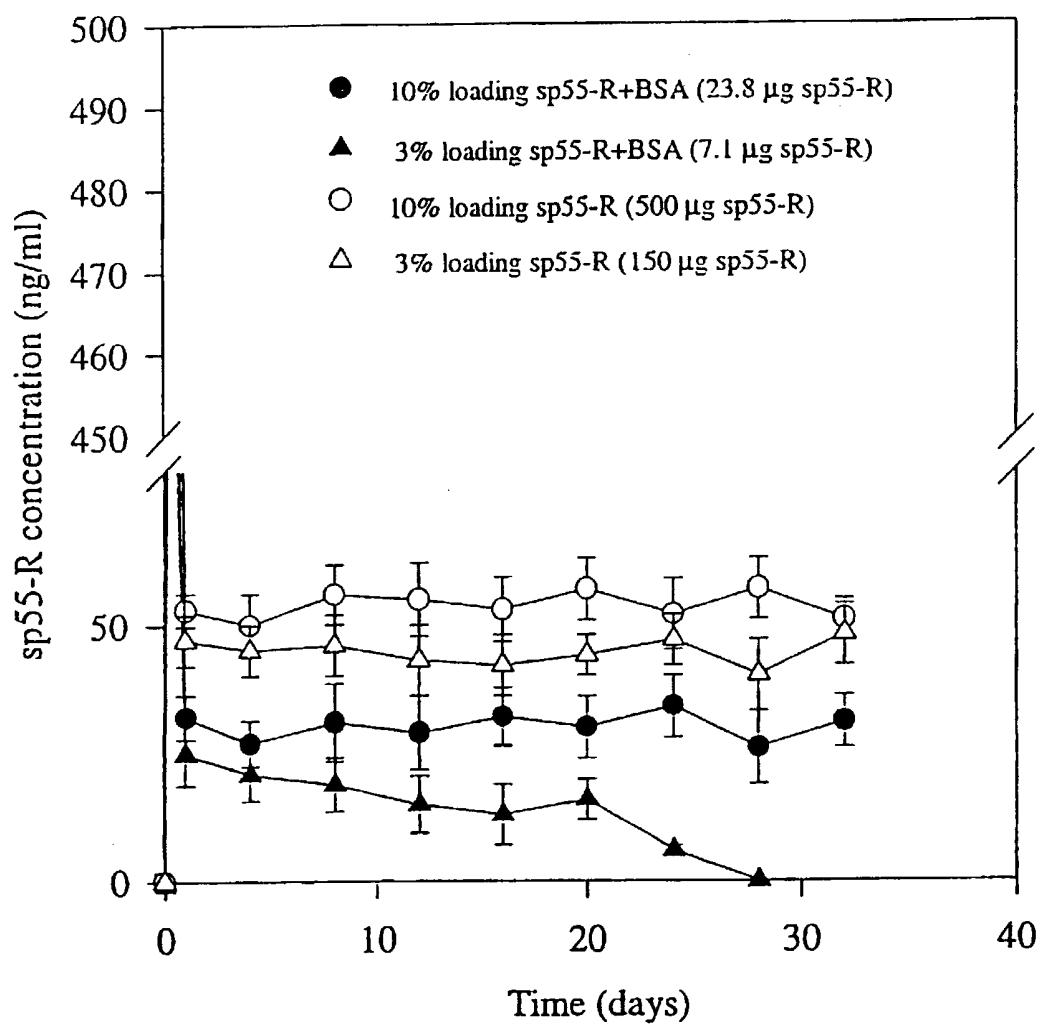
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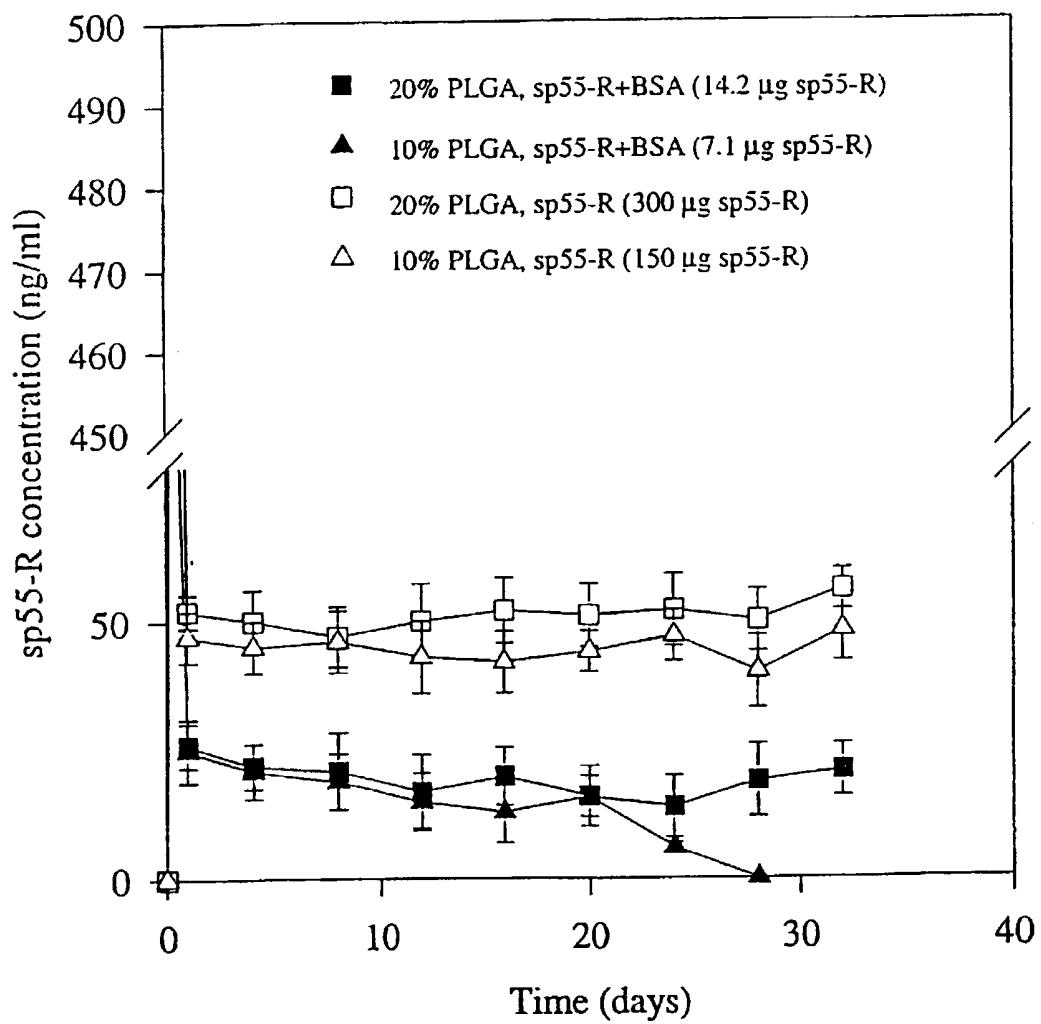
**Fig. 10B**

**Fig. 11**

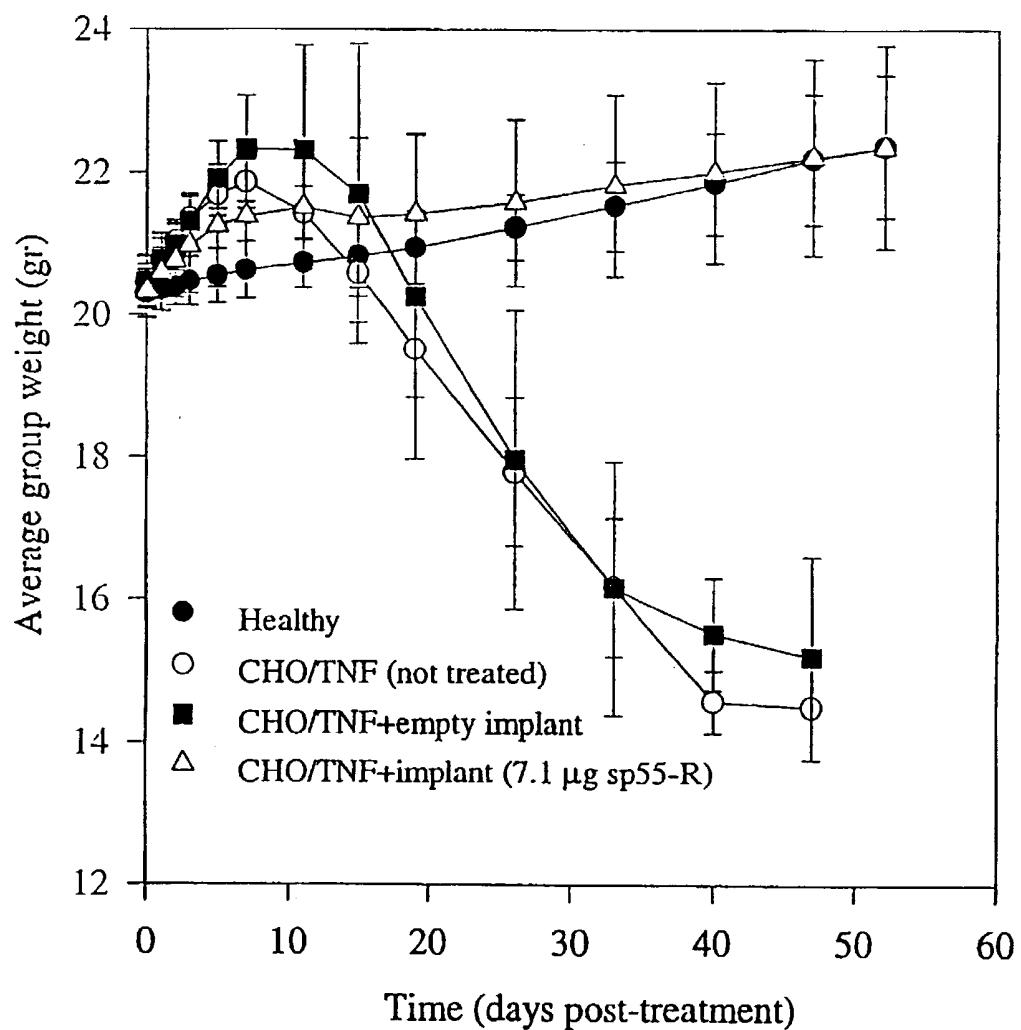
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**Fig. 12A**

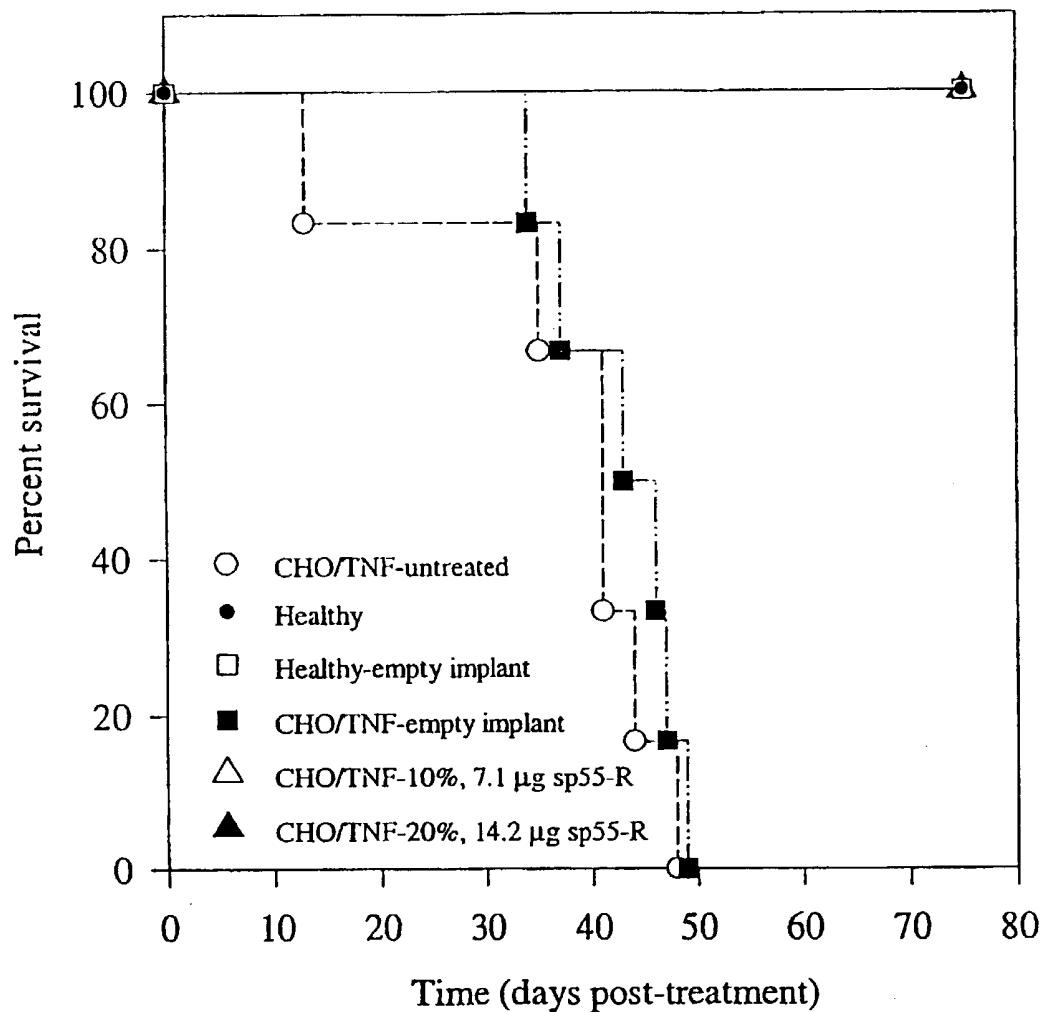
**Fig. 12B**

**Fig. 13**

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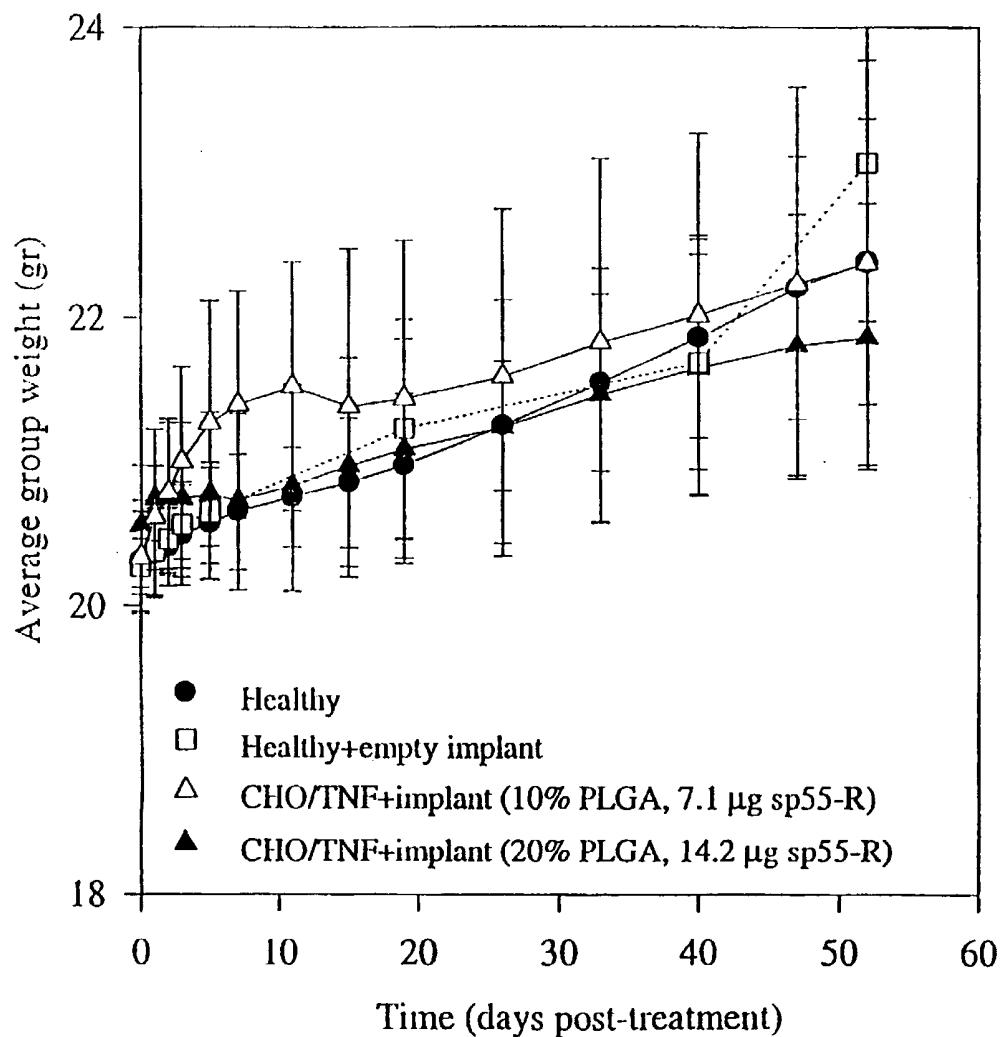
**Fig. 14**

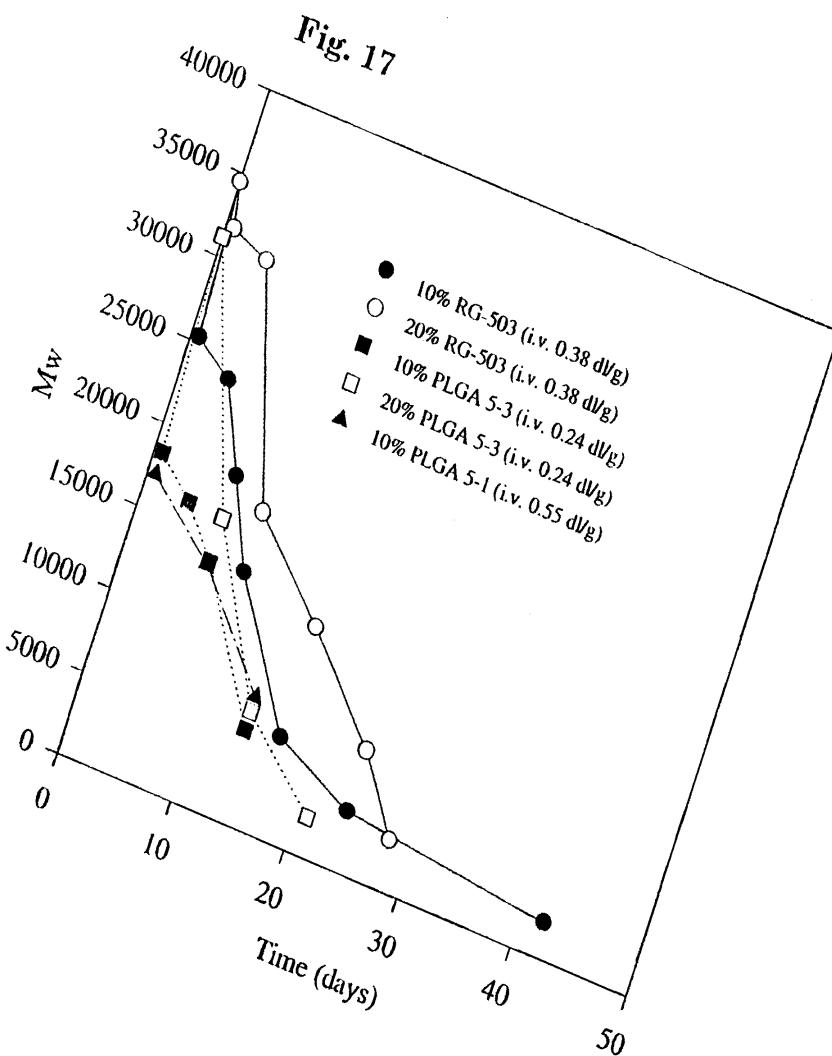
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**Fig. 15**

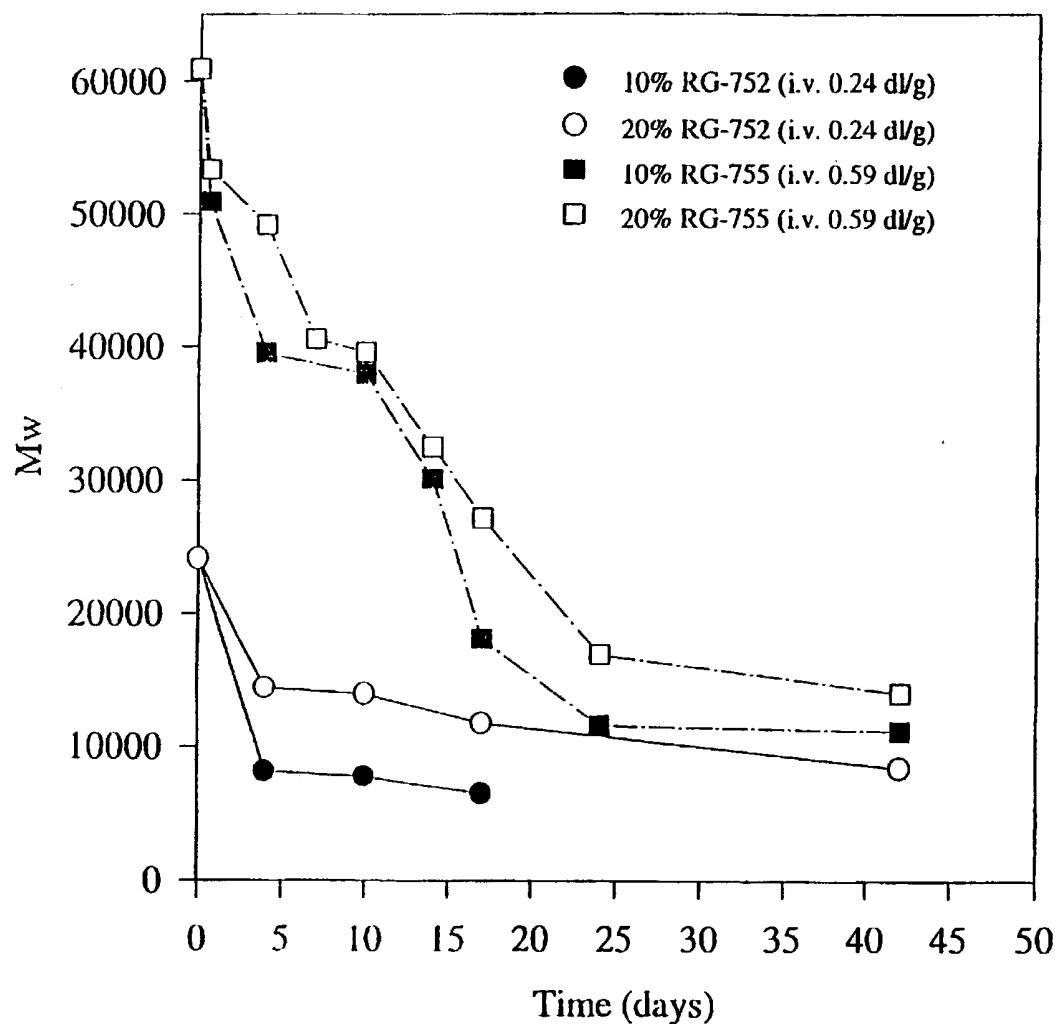
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Fig. 16





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**Fig. 18**

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL 97/00154

## A. CLASSIFICATION OF SUBJECT MATTER

**IPC6: A61L 27/00**

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

**IPC6: A61L, A61K**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## REGISTRY FILE, CAPLUS, WPI

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, A	WO 9715285 A1 (ATRIX LABORATORIES, INC.), 1 May 1997 (01.05.97), page 6, line 34, page 7, line 29 - line 33 --	1-3
A	EP 0539751 A1 (ATRIX LABORATORIES, INC.), 5 May 1993 (05.05.93), abstract, column 6, line 3 - line 18, column 9, line 9 - line 24, claims --	1-27
A	US 4938763 A (RICHARD L. DUNN ET AL), 3 July 1990 (03.07.90), abstract, column 5, line 1 - line 19, claims -----	1-27



Further documents are listed in the continuation of Box C.



See patent family annex.

- \* Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

28 August 1998

Date of mailing of the international search report

30-09- 1997

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2  
 NL-2280 HV Rijswijk  
 Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl.  
 Fax: (+ 31-70) 340-3016

Authorized officer

SOFIA NIKOLOPOULOU

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL 97/00154

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 13-22, 24-26

is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2.  Claims Nos.: because they relate to parts of the international Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

SA 1590

## INTERNATIONAL SEARCH REPORT

Information on patent family members

06/08/97

International application No.  
PCT/IL 97/00154

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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